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(71) Applicant *(for all designated States except US)*: CONNAUGHT LABORATORIES LIMITED [CA/CA]; 1755 Steeles Avenue, Toronto, Ontario M2R 3T4 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LOOSMORE, Sheena, M. [CA/CA]; 70 Crawford Rose Drive, Aurora, Ontario L4G 4R4 (CA). YANG, Yan-Ping [CA/CA]; Apt. 709, 120 Torresdale Avenue, Willowdale, Ontario M2R 3N7 (CA). KLEIN, Michel, H. [CA/CA]; 16 Munro Boulevard, Willowdale, Ontario M2P 1B9 (CA).

(74) Agent: STEWART, Michael, I.; Sim & McBurney, 6th Floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).

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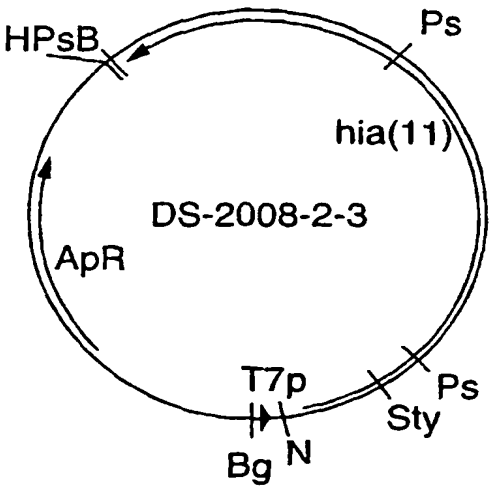
(57) Abstract

Recombinant production of Hia protein, in full-length and N-terminally truncated forms, of non-typeable strains of *Haemophilus influenzae*, is described. The nucleic acid and deduced amino acid sequences of Hia genes of various strains of non-typeable and type c *Haemophilus influenzae* also are described.

Restriction map of DS-2008-2-3, pT7 hia (11).



pT7 hia (11)

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TITLE OF INVENTION

RECOMBINANT *HAEMOPHILUS INFLUENZAE* ADHESIN PROTEINS

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending United States Patent Application No. 09/268,347.

FIELD OF INVENTION

The present invention relates to the field of molecular genetics and, in particular, to the production of recombinant *Haemophilus influenzae* adhesin (Hia) proteins.

BACKGROUND TO THE INVENTION

Haemophilus influenzae is the cause of several serious human diseases, such as meningitis, epiglottitis, septicemia and otitis media. There are six serotypes of *H. influenzae*, designated a to f, that are identified by their capsular polysaccharide. *H. influenzae* type b (Hib) was a major cause of bacterial meningitis until the introduction of several Hib conjugate vaccines in the 1980's (ref. 1. Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Vaccines based upon *H. influenzae* type b capsular polysaccharide conjugated to diphtheria toxoid (ref. 2), tetanus toxoid (ref. 3 and US patent 4,496,538), or *Neisseria meningitidis* outer membrane protein (ref. 4) have been effective in reducing *H. influenzae* type b-

induced meningitis. The other serotypes of *H. influenzae* are associated with invasive disease at low frequencies, although there appears to be an increase in the incidence in disease caused by these strains as the incidence of Hib disease declines (ref. 5; ref. 6). Non-encapsulated or non-typeable *H. influenzae* (NTHi) are also responsible for a wide range of human diseases including otitis media, epiglottitis, pneumonia, and tracheobronchitis. The incidence of NTHi-induced disease has not been affected by the introduction of the Hib vaccines (ref. 7).

Otitis media is the most common illness of early childhood, with 60 to 70% of all children, of less than 2 years of age, experiencing between one and three ear infections (ref. 8). Chronic otitis media is responsible for hearing, speech and cognitive impairments in children. *H. influenzae* infections account for about 30% of the cases of acute otitis media and about 60% of chronic otitis media. In the United States alone, treatment of otitis media costs between 1 and 2 billion dollars per year for antibiotics and surgical procedures such as tonsillectomies, adenoidectomies and insertion of tympanostomy tubes. It is estimated that an additional $30 billion is spent per annum on adjunct therapies, such as speech therapy and special education classes. Furthermore, many of the causative organisms of otitis media are becoming resistant to antibiotic treatment. An effective prophylactic vaccine against otitis media is thus desirable.

During natural infection by NTHi, surface-exposed outer membrane proteins that stimulate an antibody response are potentially important targets for bactericidal and/or protective antibodies and, therefore, potential vaccine candidates. A family of high molecular weight proteins (HMW1 and HMW2) that are important in attachment of NTHi to epithelial cells has been identified in about 70 to 75% of NTHi strains (ref. 9; ref. 10). These high molecular weight adhesins have been shown to afford some protection in the chinchilla model of otitis media (ref. 11). A second family of high molecular weight adhesion proteins has been identified in about 25% of NTHi and in encapsulated *H. influenzae* strains (ref. 12; ref. 13, ref. 14). The NTHi member of this second family is termed *Haemophilus influenzae* adhesin or Hia and the homologous protein found in encapsulated strains is termed *Haemophilus influenzae* surface fibril protein or Hsf. The *hia* gene was originally cloned from an expression library using convalescent sera from an otitis media patient, which indicates that it is an important immunogen during disease. The prototype Hia and Hsf proteins demonstrate about 82% sequence similarity, although the Hsf protein is considerably larger. The proteins are comprised of conserved amino and carboxy termini and several repeat motifs, with Hsf containing more repeat sequences than Hia. A high molecular weight protein (200 kDa) has also been identified from *Moraxella catarrhalis* that has some sequence homology with the Hsf and Hia proteins (U.S. Patent No. 5,808,024).

Since Hia or Hsf is conserved amongst encapsulated strains of *Haemophilus influenzae* and about 20 to 25% of non-encapsulated strains, and has been demonstrated to be an adhesin, the protein has utility in diagnosis of and vaccination against disease caused by *H. influenzae* or other bacterial pathogens that produce Hia or a protein capable of raising antibodies specifically reactive with Hia.

A disadvantage of Hia for use as an antigen in diagnosis, for the generation of anti-Hia antibodies useful in diagnosis and as an immunogen in vaccination is the low recovery of the native protein from *Haemophilus influenzae* species.

It would be advantageous to provide recombinant Hia protein for use as antigens, in immunogenic preparations including vaccines, carriers for other immunogens and in the generation of diagnostic reagents.

SUMMARY OF THE INVENTION

The present invention is directed towards the provision of recombinant *H. influenzae* adhesin (rHia) proteins.

In connection with the provision of such recombinant proteins, the present invention provides certain isolated and purified nucleic acid molecules. Accordingly, in one aspect thereof, the present invention provides an isolated and purified nucleic acid molecule encoding a *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* having: (a) a DNA sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21,

22, 23, 24 and 25 (SEQ ID Nos: 23, 25, 27, 29, 31, 33, 35, 37); or (b) a DNA sequence encoding a *Haemophilus influenzae* adhesin (Hia) protein having an amino acid sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 24, 26, 28, 30, 32, 34, 36, 38).

Such nucleic acid may be included in a vector, which may be a plasmid vector. In particular, the nucleic acid molecule may encode the Hia protein from strain 11 or 33 of non-typeable *Haemophilus*.

In another aspect of the present invention, there is provided an isolated and purified nucleic acid molecule encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* which is amplifiable by a pair of nucleotides which are selected from the group consisting of SEQ ID No: 7 and SEQ ID No: 15; SEQ ID No: 9 and SEQ ID No: 15; SEQ ID No: 11 and SEQ ID No: 15; SEQ ID No: 13; SEQ ID No: 15; SEQ ID No: 49; and SEQ ID No: 51.

Such nucleic acid may be included in a vector, which may be a plasmid vector. In particular, the nucleic acid molecule may encode an N-truncated Hia protein from strain 11 or 33 of non-typeable *Haemophilus*, starting at codon V38 or S44.

The plasmid vector incorporating the isolated and purified nucleic acid provided in accordance with these aspects of the invention may have the identifying characteristics of a plasmid which is selected from the group consisting of:

DS-2008-2-3 as shown in Figure 1A

DS-2186-1-1 as shown in Figure 5A

DS-2201-1 as shown in Figure 5A

DS-2186-2-1 as shown in Figure 5A

DS-2168-2-6 as shown in Figure 5A

1A-191-3-1 as shown in Figure 32

The vector provided herein may include the *cer* gene from *E. coli*. Accordingly, in another aspect of the present invention, there is provided a vector for transforming a host, comprising a nucleic acid molecule encoding a full-length or N-truncated *Haemophilus influenzae* adhesin (Hia) protein, a promoter for expression of said full-length or truncated Hia protein and, optionally, the *cer* gene of *E. coli*. The vector may be a plasmid vector or other non-replicating vector, which may have the identifying characteristics of a plasmid vector which is selected from the group consisting of:

BK-96-2-11 as shown in Figure 6A

DS-2242-1 as shown in Figure 7A

DS-2242-2 as shown in Figure 7A

DS-2340-2-3 as shown in Figure 8A

DS-2447-2 as shown in Figure 9A

DS-2448-17 as shown in Figure 9B

JB-2930-3 as shown in Figure 32

The vectors provided herein may comprise a replicating vector, including a vector from *Salmonella*, BCG, adenovirus, poxvirus, vaccinia or poliovirus.

Any of the vectors provided herein may be employed to transform a suitable host cell for expression therein of a protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus*,

which may be in full-length or truncated form. Such host conveniently may be *E. coli*. Such expression may be under the control of the T7 promoter and expression of the recombinant Hia from the transformed host may be effected by culturing in an inducing concentration of lactose or other convenient inducing agent.

The present invention further includes, in a further aspect thereof, a recombinant protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable *Haemophilus* strain producible by the transformed host, particularly *E. coli*, provided herein. Such Hia protein may be provided in the form of an immunogenic fragment or adhesin-functional analog of the recombinant protein.

The recombinant Hia proteins, full-length or N-truncated, provided herein are useful as antigens in immunogenic compositions, carriers for other immunogens, diagnostic agents and in the generation of diagnostic agents. The nucleic acid molecules which encode the Hia protein, full-length or N-truncated, also are useful as probes for diagnostic use and also in immunogenic compositions.

The present invention, in an additional aspect thereof, provides an immunogenic composition, comprising at least one immunologically active component which is selected from the group consisting of an isolated and purified nucleic acid molecule as provided herein and a recombinant protective Hia protein, full-length or N-truncated, of a strain of *Haemophilus*, as provided herein, and a pharmaceutically-acceptable carrier therefor.

The immunogenic compositions provided herein may be formulated as a vaccine for *in vivo* administration to a host to provide protection against disease caused by *H. influenzae*. For such purpose, the compositions may be formulated as a microparticle, capsule, ISCOM or liposome preparation. The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces.

The immunogenic compositions of the invention (including vaccines) may further comprise at least one other immunogenic or immunostimulating material and the immunostimulating material may be at least one adjuvant or at least one cytokine. Suitable adjuvants for use in the present invention include (but are not limited to) aluminum phosphate, aluminum hydroxide, QS21, Quil A, derivatives and components thereof, ISCOM matrix, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octadecyl ester of an amino acid, a muramyl dipeptide, polyphosphazene, ISCOPREP, DC-chol, DDBA and a lipoprotein and other adjuvants.

Advantageous combinations of adjuvants are described in copending United States Patent Application Serial No. 08/261,194 filed June 16, 1994 and 08/483,856 filed June 7, 1995, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference (WO 95/34308 published November 21, 1995).

In accordance with another aspect of the invention, there is provided a method for generating an immune response in a host, comprising the step of

administering to a susceptible host an effective amount of the immunogenic composition as recited above. The immune response may be humoral or a cell-mediated immune response. Hosts in which protection against disease may be conferred include primates, including humans.

In accordance with other aspects of the invention, there is provided the immunogenic compositions provided herein when used as a medicament and the use of these components of the immunogenic compositions in the manufacture of an immunogenic composition.

The present invention includes, in a yet additional aspect thereof, a method for the production of a protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus influenzae*, which comprises:

transforming a host, such as *E. coli*, with a vector comprising a nucleic acid molecule encoding an N-truncated form of the *Haemophilus influenzae* adhesin protein as provided herein,

growing the host to express the encoded truncated Hia, and

isolating and purifying the expressed Hia protein.

The encoded truncated Hia may be expressed in inclusion bodies. The isolation and purification step may be effected by disrupting the grown transformed cells to produce a supernatant and the inclusion bodies containing the Hia, solubilizing the inclusion bodies after separation from the supernatant, to produce a solution of the recombinant Hia, chromatographically purifying the solution of recombinant Hia free from

cell debris, and isolating the purified recombinant Hia protein.

The vector transforming the host cell, such as *E. coli*, may include the T7 promoter and the *E. coli* or other host cell may be cultured in the presence of an inducing amount of lactose or other convenient inducing agent.

The strain of *Haemophilus influenzae* herein may be selected from the group of non-typeable strains consisting of strains 11, 33, 32, 29, M4071, K9, K22 and 12. Specific nucleic acid sequences for the genes encoding the respective Hia proteins from such strains are provided herein and are described below.

The nucleic acid molecules provided herein are useful in diagnostic applications. Accordingly, in a further aspect of the invention, there is provided a method of determining the presence, in a sample, of nucleic acid encoding a *Haemophilus influenzae* adhesin protein, comprising the steps of:

a) contacting the sample with a nucleic acid molecule as provided herein to produce duplexes comprising the nucleic acid molecule provided herein are nucleic acid encoding the Hia protein of a strain of *Haemophilus* present in the sample and specifically hybridizable therewith; and

b) determining the production of the duplexes.

In addition, the present invention provides a diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a *Haemophilus influenzae* adhesin protein, comprising:

a) a nucleic acid molecule as provided herein;

b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising the nucleic acid molecule and any such nucleic acid molecule; and

c) means for determining production of the duplexes.

The recombinantly produced truncated Hia proteins provided herein also are useful in diagnostic applications. Accordingly, in another aspect of the invention, there is provided a method of determining the presence of antibodies specifically reactive with the Hia protein in a sample, comprising the steps of (a) contacting the sample with the recombinant Hia protein provided herein to provide complexes of the recombinant Hia protein and any such antibodies present in the sample specifically reactive therewith; and (b) determining production of the complexes.

Advantages of the present invention include:

- an isolated and purified nucleic acid molecule encoding a *Haemophilus influenzae* adhesin protein or a fragment or an analog of the Hia protein;
- recombinantly-produced Hia proteins, free from any other *Haemophilus* proteins; and
- diagnostic kits and immunological reagents for specific identification of *Haemophilus*.

BRIEF DESCRIPTION OF DRAWINGS

The present invention will be further understood from the following description with reference to the drawings, in which:

Figure 1A shows a restriction map for plasmid DS-2008-2-3 that contains the T7 promoter and the full-length NTHi strain 11 *hia* gene.

Figure 1B shows the oligonucleotides used to PCR amplify the strain 11 *hia* gene. Sense Strand (5038.SL): SEQ ID No: 1, encoded amino acids SEQ ID No: 2; Antisense Strand (5039.SL): SEQ ID No: 3, complement SEQ ID No: 4, encoded amino acids SEQ ID No: 5. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; N, *Nde* I; Ps, *Pst* I; Sty, *Sty* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance.

Figure 2 shows an immunoblot of the recognition of full-length rHia protein by anti-native *Moraxella catarrhalis* high molecular weight adhesin antibody. Lane 1, DS-2043-1 uninduced; lane 2, DS-2043-1, induced for 4h; lane 3, DS-2043-2 uninduced; lane 4, DS-2043-2, induced for 4h; lane 5, molecular weight markers. DS-2043-1 and DS-2043-2 are independent clones of *pT7 hia(11)* in BL21 (DE3).

Figure 3 shows the construction of plasmids DS-2092-1 and DS-2092-40 that contain tandem copies of the *T7 hia* gene cassette for the strain 11 *hia* gene. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; Ps, *Pst* I; Xb, *Xba* I. Other abbreviations are: CAP, calf alkaline phosphatase; T7p, T7 promoter; ApR, ampicillin resistance.

Figure 4 shows the sites of truncation for the strain 11 Hia protein (SEQ ID No: 6).

Figure 5A shows the construction of plasmids expressing truncated *hia* genes from strain 11.

Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; N, *Nde* I; Nhe, *Nhe* I; Ps, *Pst* I; R, *EcoR* I; Sty, Sty I; Xb, *Xba* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance.

Figure 5B shows the oligonucleotides used to PCR amplify the 5'-fragments for the truncated genes. E21 truncation: Sense (5524.SL): SEQ ID No: 7, encoded amino acids SEQ ID No: 8; T33 truncation: Sense (5525.SL) SEQ ID No: 9, encoded amino acids SEQ ID No: 10; V38 truncation: Sense (5526.SL): SEQ ID No: 11, encoded amino acids, SEQ ID No: 12; N52 truncation: Sense (5527.SL): SEQ ID No: 13, encoded amino acids SEQ ID No: 14; Antisense (5528.SL): SEQ ID No: 15; complement SEQ ID No: 16, encoded amino acids SEQ ID No: 17.

Figure 6A shows the construction of plasmid BK-96-2-11 that contains the V38 *hia* gene from NTHi strain 11 and the *E. coli cer* gene. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; K, *Kpn* I; N, *Nde* I; P, *Pst* I; R, *EcoR* I; S, *Sal* I; Sm, *Sma* I; Sty, *Sty* I; Xb, *Xba* I; Xho, *Xho* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; CAP, calf alkaline phosphatase; tt1 transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 6B shows the oligonucleotides used to construct the multiple cloning site and transcription terminators. "R" and "Ps" indicate termini that will overlap with EcoR I or Pst I ends, but will not re-

generate the sites. Upperstrand (SEQ ID No.: 50) lower strand (SEQ ID No.: 51).

Figure 7A shows the construction of plasmids DS-2242-1 and DS-2242-2 that contain the T7 promoter and full-length NTHi strain 33 *hia* gene, the *E. coli cer* gene and the kanamycin resistance gene. Restriction enzyme sites are: A, *AlwN* I; B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; K, *Kpn* I; N, *Nde* I; Ps, *Pst* I; R, *EcoR* I; S, *Sal* I; Sm, *Sma* I; Xb, *Xba* I; Xho, *Xho* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; tt1, transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 7B shows the oligonucleotides used to generate the 5'-end of the strain 33 *hia* gene coding strand (SEQ ID No.: 52), complementary strand (SEQ ID No.: 53), and encoded amino acid sequence (SEQ ID No.: 54).

Figure 8A shows the construction of plasmid DS-2340-2-3 that contains the T7 promoter and the V38 *hia* gene from strain 33, the *E. coli cer* gene and the kanamycin resistance gene. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; N, *Nde* I; Ps; *Pst* I; R, *EcoR* I; S, *Sal* I; Sn, *SnaB* I; Xb, *Xba* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; tt1, transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 8B shows the oligonucleotides used to PCR amplify the 5'-end of the truncated *hia* gene. Sense (6286.SL): SEQ ID No: 16, encoded amino acids SEQ ID

No: 17; antisense (6287.SL) SEQ ID No: 18, complement SEQ ID No: 19, encoded amino acids SEQ ID No: 20.

Figures 9A and 9B show the construction of plasmids DS-2447-2 and DS-2448-17, that contain tandem copies of the *T7 V38 hia (11)* and *T7 V38 hia (33)* genes, respectively. Restriction enzyme sites are: B, BamH I; Bg, Bgl II; H, Hind III; Ps; Pst I; R, EcoR I; S, Sal I; Xb, Xba I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; CAP, calf alkaline phosphatase; tt1, transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 10 shows the expression of rHia. Panel A: lane 1, full-length rHia (11) no induction; lane 2, full-length rHia (11); lane 3, E21 rHia (11); lane 4, T33 rHia (11); lane 5, V38 rHia (11); lane 6, N52 rHia (11). Panel B: lane 1, V38 rHia (11) no induction; lane 2, V38 rHia (11); lane 3, V38 rHia (11)/cer.

Figure 11 shows a purification scheme for rHia proteins. Abbreviations are: SP, supernatant; PPT, precipitate; DTT, dithiothreitol; OG, octyl glucoside; (x) means discarded.

Figure 12, having panels A and B, shows the SDS-PAGE analysis of purified rHia. Panel A shows purified V38 rHia protein from strain 11 and panel B shows purified V38 rHia protein from strain 33. Lane 1, molecular weight markers; lane 2, whole-cell lysate; lane 3, crude extract; lane 4, purified rHia protein.

Figure 13, having panels A, B and C, shows the stability of V38 rHia (11). Panel A shows samples stored at 4°C without glycerol. Panel B shows samples

stored at 4°C, in the presence of 20% glycerol. Panel C shows samples stored at -20°C in the presence of 20% glycerol. Lane 0 indicates t_0; lanes 1 to 8 indicate samples stored for 1 to 8 weeks.

Figure 14, having panels A and B, shows the immunogenicity of V38 rHia (11) or V38 rHia (33) in CD-1 mice. Panel A shows the response after a single immunization and panel B shows the response of a prime/boost immunization.

Figures 15A and 15B show the immunogenicity of V38 rHia (11) in BALB/c mice and guinea pigs. Figure 15A shows the antibody response in mice and Figure 15B shows the response in guinea pigs.

Figure 16 illustrates the protective ability of V38 rHia (33) against nasopharyngeal colonization in a chinchilla model.

Figure 17 shows the oligonucleotides used to PCR amplify additional *hia* genes. Sense (5040.SL), SEQ ID No: 21, encoded amino acids SEQ ID No: 22; Antisense (5039.SL), SEQ ID No: 3, complement SEQ ID No: 4, encoded amino acids SEQ ID No: 5.

Figure 18 shows the nucleotide sequence (SEQ ID No: 23) and deduced amino acid sequence (SEQ ID No: 24) of the *hia* gene from NTHi strain 33.

Figure 19 shows the nucleotide sequence (SEQ ID No: 25) and deduced amino acid sequence (SEQ ID No: 26) of the hia gene from NTHi strain 32.

Figure 20 shows the nucleotide sequence (SEQ ID No: 27) and deduced amino acid sequence (SEQ ID No: 28) of the *hia* gene from NTHi strain 29.

Figure 21 shows the nucleotide sequence (SEQ ID No: 29) and deduced amino acid sequence (SEQ ID No: 30 of the *hia* gene from NTHi strain M4071.

Figure 22 shows the nucleotide sequence (SEQ ID No: 31) and deduced amino acid sequence (SEQ ID No: 32) of the *hia* gene from NTHi strain K9.

Figure 23 shows the nucleotide sequence (SEQ ID No: 33) and deduced amino acid sequence (SEQ ID No: 34) of the *hia* gene from NTHi strain K22.

Figure 24 shows the nucleotide sequence (SEQ ID No: 35) and deduced amino acid sequence (SEQ ID No: 36) of the *hia* gene from type c strain API.

Figure 25 shows the nucleotide sequence (SEQ ID No: 37) and deduced amino acid sequence (SEQ ID No: 38) of the *hia* locus from NTHi strain 12. The overlined or underlined sequences indicate oligonucleotides used to PCR amplify across the junction of the two *orfs*. Sense (6431.SL) SEQ ID No: 39, (6432.SL) SEQ ID No: 40; antisense (6295.SL) SEQ ID No: 41, (6271.SL) SEQ ID No: 42.

Figure 26 shows the nucleotide sequence (SEQ ID No.: 43) and deduced amino acid sequence (SEQ ID No.: 44) of the *hia* locus from NTHi strain 11, as published in U.S. Patent No. 5,646,259.

Figure 27 shows the alignment of the upstream ORF from the strain 12 *hia* locus (SEQ ID No: 45) with part of the HI1732 protein (SEQ ID No: 46) from *H. influenzae* type b strain Rd.

Figure 28 shows the alignment of amino acid sequences from Hia (SEQ ID Nos. 24, 26, 28, 34, 30, 44, 32), Hsf (SEQ ID No.: 47) and partial sequences from

Moraxella catarrhalis high molecular weight proteins (200 kDa) from strains 4223 and LES-1 (SEQ ID Nos.: 48, 49). Asterisks within sequences indicate stop codons, but below the sequence they indicated sequence homology. Dots indicate identical residues. The sequence alignments were prepared by direct comparison of the amino acid sequences of the respective proteins.

Figure 29 shows the oligonucleotides used to PCR amplify the 5' end of the hia gene at the S44 truncated position. Sense (6817.SL) SEQ ID No: 49, encoding amino acids SEQ ID No: 50; antisense (6818.SL) SEQ ID No: 51, complement SEQ ID No: 52, encoded amino acids SEQ ID No: 53.

Figure 30 shows the construction of plasmid JB-2930-3 that contains the S44 *hia* gene from NTHi strain 11 and the E. coli *cer* gene and the T7 promoter. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; K, *Kpn* I; N, *Nde* I; P, *Pst* I; R, *EcoR* I; S, *Sal* I; Sm, *Sma* I; Sty, *Sty* I; Xb, *Xba* I; Xho, *Xho* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; CAP, calf alkaline phosphatase; tt1 transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 31 shows SDS-PAGE analysis of the expression of rHia from S44. Lane 1, expression from pET S44 vector at time 0 (no induction); lane 2 expression from pET S44 vector after 4 hours induction; lane 3 expression from JB-2930-3 after 4 hours induction.

Figures 32 shows a schematic representation of the two vectors used for the expression study, JB-2930-3 and IA-191-3-1, of S44-truncated rHia.

GENERAL DESCRIPTION OF THE INVENTION

Since *H. influenzae* strains produce low quantities of the Hia and Hsf proteins, the *hia* gene from NTHi strains was cloned into an expression vector for overproduction of the recombinant protein in *E. coli*. When the full-length recombinant Hia (rHia) protein was expressed, it was made in relatively low quantities. In order to confirm that there was expression of the recombinant protein, an immunoblot was performed using antibody raised to a *Moraxella catarrhalis* high molecular weight adhesin protein identified as 200 kDa in US Patent No. 5,808,024, assigned to the assignee and the disclosure of which is incorporated herein by reference. Antibody against the gel-purified native 200 kDa protein recognized a specific induced band in the rHia protein sample. The yield of rHia was not significantly improved by increasing the gene copy number of the T7 *hia* gene cassette.

The *E. coli cer* gene has been shown to stabilize plasmids containing large inserts (ref. 15), but the yield of rHia was not significantly improved by adding the *E. coli cer* gene to the expression vector. However, the *E. coli* cells were observed to clump during culture, suggesting that there was surface expression of the Hia adhesin protein. The apparent toxicity of the rHia protein might be overcome if it were made as inclusion bodies, so truncations were made at the 5'-end of the gene to delete putative signal

sequences. This modification resulted in good production and recovery of truncated rHia starting from the V38 position.

The full-length and V38-truncated rHia proteins were immunogenic and the resultant anti-rHia antibodies were protective in passive infant rat models of bacteremia due to *H. influenzae* type a or type b strains. In addition, the truncated V38 rHia protein was found to be partially protective against nasopharyngeal colonization in an active challenge model in chinchillas. The protection afforded by rHia derived from an NTHi strain against disease caused by NTHi and encapsulated type a or type b strains, indicates that there may be common protective epitopes. The cloning and sequence analysis of additional *hia* genes may help to identify conserved regions. The full-length or N-terminal truncated rHia proteins may be used as vaccine components to protect against *Haemophilus influenzae* disease.

Any *Haemophilus* strains that have *hia* genes may be conveniently used to provide the purified and isolated nucleic acid molecules (which may be in the form of DNA molecules), comprising at least a portion coding for a Hia protein as typified by embodiments of the present invention. Such strains are generally available from clinical sources and from bacterial culture collections, such as American Type Culture Collection. Appropriate strains of *Haemophilus* include:

Non-typeable *Haemophilus* strain 11;

Non-typeable *Haemophilus* strain 33;

Non-typeable *Haemophilus* strain 32;

Non-typeable *Haemophilus* strain 29;

Non-typeable *Haemophilus* strain M4071;

Non-typeable *Haemophilus* strain K9;

Non-typeable *Haemophilus* strain K22;

Non-typeable *Haemophilus* strain 12;

Type C *Haemophilus* strain API.

In this application, the term "Hia" protein is used to define a family of Hia proteins that includes those having naturally occurring variations in their amino acid sequences as found in various strains of *Haemophilus*.

Referring to Fig. 1A, there is illustrated a restriction map of plasmid DS-2008-2-3 that contains a full-length *hia* gene from non-typeable *Haemophilus influenzae* strain 11, under the influence of the T7 promoter. The nucleic acid (SEQ ID No.: 43) and deduced amino acid sequence (SEQ ID No.: 44) of the *hia* gene from strain 11, are described in the aforementioned U.S. Patent No. 5,646,259 (and identified therein as "HA1"). The oligonucleotides used to PCR amplify the *hia* gene from the ATG start codon of the gene of strain 11 are shown in Fig. 1B.

Referring to Fig. 2, there is illustrated an immunoblot demonstrating the recognition of the rHia (11) protein by anti-native *Moraxella catarrhalis* high molecular weight adhesin antibody. The *M. catarrhalis* high molecular weight adhesin or 200 kDa protein described in the aforementioned US Patent No. 5,808,024 has some sequence homology with the Hia and Hsf proteins, especially at the carboxy terminus (Fig. 2B).

Referring to Fig. 3, there is illustrated a construction scheme for plasmids DS-2092-1 and DS-2092-40 that contain tandem copies of T7 *hia* gene cassettes comprising the full-length *hia* gene from NTHi strain 11. Such plasmids that contain increased copy numbers of genes often have enhanced production levels for recombinant proteins. However, as seen below, the low yield of recombinant Hia was not significantly improved by increasing the gene copy number.

Referring to Fig. 4, there is illustrated the N-terminal sequence of the NTHi strain 11 protein and the position of time N-terminally truncated rHia proteins. The N-terminal truncation up to position E21 deletes a long hydrophobic region that may constitute part of a signal sequence for Hia. The deletion up to position T33 includes a long hydrophobic region and follows a potential Ala-X-Ala signal cleavage site. The deletion up to position V38 includes a long hydrophobic region and follows a potential Ala-X-Ala signal cleavage site. The recombinant Hia protein starting at position S44 includes a long hydrophobic region and follows a potential Ala-X-Ala signal cleavage site. The recombinant Hia protein starting at position N52 mimics the approximate start of the related high molecular weight (200 kDa) adhesin from *Moraxella catarrhalis* described in the aforementioned US Patent No. 5,808,024, which recombinant protein is over-produced if truncated at its N-terminus to start at V56.

Referring to Fig. 5A, there is illustrated the construction scheme for the generation of plasmids DS-2186-1-1, DS-2201-1, DS-2186-2-1, and DS-2168-2-6

producing four of the N-terminal truncated rHia proteins. The oligonucleotides used to PCR amplify the 5'-fragments are shown in Fig. 5B. In Figure 30, there is illustrated the construction scheme for the generation of plasmids JB-2930-3, which produces the S44 deletion. The oligonucleotides used to PCR amplify the 5'-fragments are shown in Figure 29.

Referring to Fig. 6A, there is illustrated a construction scheme for the generation of plasmid BK-96-2-11 that contains the V38 *hia* gene from NTHi strain 11 as well as the *E. coli cer* gene that has been shown to stabilize plasmids. The introduction of the *cer* gene into plasmids producing toxic proteins, was predicted to enhance protein production. There was an observed change in the morphology of the *E. coli* cells producing full-length rHia in the presence of the *cer* gene, in that they clumped. This suggests that there was enhanced expression of the adhesin at the surface of the cells that caused the clumping. The expression plasmid BK-96-2-11 also contains transcription terminators upstream and downstream of the T7 V38 *hia* gene cassette that were predicted to enhance the gene stability. The oligonucleotides used to generate the multiple cloning site and transcription terminators are shown in Fig. 6B.

Referring to Fig. 7A, there is illustrated a construction scheme for plasmids DS-2242-1 and DS-2242-2 that contain a full-length *hia* gene from non-typeable *Haemophilus influenzae* strain 33, under the influence of the T7 promoter. The expression plasmids also contain the *E. coli cer* gene and transcription

terminators upstream and downstream of the T7 *hia* (33) gene cassette. DS-2242-1 has the terminators coded on the same strand as the T7 *hia* (33) gene. However, there was no observable difference in the expression of rHia from the two plasmids. The oligonucleotides used to construct the authentic 5'-end of the NTHi strain 33 gene are shown in Fig. 7B.

Referring to Fig. 8A, there is illustrated a construction scheme for plasmid DS-2340-2-3 that contains the V38 *hia* gene from NTHi strain 33 as well as the *E. coli cer* gene. There are also transcription terminators located upstream and downstream of the T7 V38 *hia* gene cassette, on the same strand. The oligonucleotides used to PCR amplify the NTHi strain 33 *hia* gene from the V38 codon, are shown in Fig. 8B.

Referring to Fig. 9, there is shown the construction of plasmids DS-2447-2 and DS-2448-17 that contain tandem copies of the *T7 V38 hia (11)* or *T7 V38 hia (33)* gene cassettes, respectively.

Referring to Fig. 10, panel A, there is illustrated the production of rHia proteins from plasmids encoding full-length or truncated *hia* genes from NTHi strain 11. The production of the full-length rHia (11) protein was very low. There was also low expression observed for the E21 and T33 truncated rHia proteins. However, the V38 and N52 truncated rHia proteins have significantly improved expression levels. As shown in Fig. 10, panel B, the production of V38 rHia (11) appears to be enhanced when the *E. coli cer* gene is added to the expression plasmid.

Referring to Fig. 11, there is illustrated a purification scheme for rHia proteins, produced as inclusion bodies. Cells were lysed by sonication and the inclusion bodies purified by serial extractions. The inclusion bodies were solubilized in guanidinium chloride and impurities precipitated by the addition of polyethlyene glycol (PEG). Addition of $(NH_4)_2SO_4$ resulted in precipitation of rHia and the crude rHia was further purified by gel filtration.

Referring to Fig. 12, there is illustrated the purified V38 rHia proteins from strains 11 and 33. The inclusion bodies are shown in lane 3 and the final purified protein in lane 4. The estimated purity of the purified protein is greater than about 90% as determined by SDS-PAGE densitometry.

Referring to Fig. 13, there is shown the SDS-PAGE analysis of the stability of rHia proteins produced as described herein during 8 weeks of storage with or without glycerol at 4°C and with glycerol at -20°C. The protein is stable under any of these conditions.

Referring to Fig. 14, there is illustrated the immunogenicity of V38 rHia proteins from strains 11 and 33 in CD-1 mice. At doses from 0.3 to 10 µg, there is a strong immune response after one or two doses with either protein. There is no obvious dose response at these levels. Similar results were observed in BALB/c mice (Fig. 15A) and in guinea pigs (Fig. 15B), indicating that rHia was very immunogenic, even at 0.3 µg per dose.

Referring to Fig. 16, there is illustrated the protection afforded by V38 rHia (33) against

colonization by NTHi strain 33. As described by Yang et al (ref. 20), a chinchilla nasopharyngeal colonization model has been developed to assess protection against this earliest stage of disease. The model was initially established for NTHi strains that express *hmw* genes and had to be adapted for NTHi strains expressing *hia* genes. For the prototype *hmw*-expressing strain (NTHi 12), 10^2 to 10^8 cfu could be used to establish infection, but 5 x 10^8 cfu of NTHi strain 33 was required, and even at this high level no infection could be established with the prototype *hia*-expressing strain 11. At a 100 μg dose, it is evident that there is partial protection in the immunized cohort, although there is no protection at a 50 μg dose. Such protection against the early stages of disease illustrates the utility of the rHia adhesins as vaccine antigens.

Referring to Fig. 17, there is illustrated the oligonucleotides used to PCR amplify additional *Haemophilus influenzae hia* genes. The sequences are based upon the conserved amino and carboxy terminal sequences of the Hia and Hsf proteins.

Referring to Fig. 18, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the NTHi strain 33 *hia* gene. Referring to Fig. 19, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the NTHi strain 32 *hia* gene. Referring to Fig. 20, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the NTHi strain 29 *hia* gene. Referring to Fig. 21, there is illustrated the

complete nucleotide sequence and deduced amino acid sequence of the NTHi strain M4071 *hia* gene. Referring to Fig. 22, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the NTHi strain K9 *hia* gene. Referring to Fig. 23, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the NTHi strain K22 *hia* gene. Referring to Fig. 24, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the *Haemophilus influenzae* type c strain API *hia* gene. Referring to Fig. 25, there is illustrated the complete nucleotide sequence and deduced amino acid sequence of the *hia* locus from NTHi strain 12. The PCR amplified fragment contains the 3'-end of a gene related to HI1733 gene of the *Haemophilus influenzae* type d strain Rd genome joined to the 3'-end of an *hia* gene. An alignment of the upstream ORF with the HI1733 protein is shown in Fig. 27.

Figure 26 shows the complete nucleotide sequence and the deduced amino acid sequence of the *Hia* gene from NTHi strain 11, as published in the aforementioned USP 5,646,259.

Referring to Fig. 28, there is illustrated an alignment of the deduced protein sequences from Hsf, Hia, and partial sequences of the *M. catarrhalis* 200 kDa protein.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have use in applications in the fields of vaccination, diagnosis, treatment of *Haemophilus* infection and the generation of immunological agents. A further non-

limiting discussion of such uses is further presented below.

Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from immunogenic recombinant *Haemophilus influenzae* adhesin (rHia) proteins of non-typeable *Haemophilus* strains, immunogenic analogs and fragments thereof and/or immunogenic peptides as disclosed herein. The vaccine elicits an immune response which produces antibodies, including anti-rHia antibodies and antibodies that are opsonizing or bactericidal.

Immunogenic compositions, including vaccines, may be prepared as injectables, as liquid solutions or emulsions. The rHia protein, immunogenic analogs and fragments thereof and/or immunogenic peptides may be mixed with pharmaceutically acceptable excipients which are compatible with the rHia protein, immunogenic fragments analogs or immunogenic peptides. Such excipients may include, water, saline, dextrose, glycerol, ethanol and combinations thereof.

The immunogenic compositions and vaccines may further contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness of the vaccines.

Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously or intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the

immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes.

The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. Some such targeting molecules include vitamin B12 and fragments of bacterial toxins, as described in WO 92/17167 (Biotech Australia Pty. Ltd.), and monoclonal antibodies, as described in U.S. Patent No. 5,194,254 (Barber et al).

Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the rHia protein, fragment analogs and/or peptides.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of

active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the rHia, analogs and fragments thereof and/or peptides. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the vaccine may also depend on the route of administration and will vary according to the size of the host.

The nucleic acid molecules encoding the rHia proteins of non-typeable *Haemophilus* may also be used directly for immunization by administration of the DNA directly, for example by injection for genetic immunization or by constructing a live vector, such as *Salmonella*, BCG, adenovirus, poxvirus, vaccinia or poliovirus, containing the nucleic acid molecule. A discussion of some live vectors that have been used to carry heterologous antigens to the immune system is contained in, for example, O'Hagan (1992) (ref. 16). Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al., 1993 (ref. 17).

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as an 0.05 to 1.0 percent solution in phosphate - buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to

produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include the specific adjuvants detailed above as well as saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvants, bacterial products, such as muramyl

dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants include:

(1) lack of toxicity;

(2) ability to stimulate a long-lasting immune response;

(3) simplicity of manufacture and stability in long-term storage;

(4) ability to elicit both CMI and HIR to antigens administered by various routes, if required;

(5) synergy with other adjuvants;

(6) capability of selectively interacting with populations of antigen presenting cells (APC);

(7) ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

US Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989 which is incorporated herein by

reference thereto teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. 1991 (ref. 18) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycosphingolipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Patent No. 4,258,029 granted to Moloney, assigned to the assignee hereof and incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al. 1990 (ref. 19), reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.

Immunoassays

The rHia protein of a non-typeable strain of *Haemophilus*, analogs and fragments thereof produced according to the present invention are useful as immunogens, as antigens in immunoassays including enzyme-linked immunosorbent assay (ELISA), RIAs and

other non-enzyme linked antibody binding assays or procedures known in the art for the detection of anti-bacterial, *Haemophilus*, and/or Hia antibodies. In ELISA assays, the Hia protein, analogs and fragments are immobilized onto a selected surface, for example a surface capable of binding proteins or peptides, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed Hia protein, analogs and/or fragments, a nonspecific protein such as a solution of bovine serum albumin (BSA) or casein that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents, such as BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to about 4 hours, at temperature such as of the order of about 25° to about 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution such as PBS/Tween, or a borate buffer.

Following formation of specific immunocomplexes between the test sample and the bound Hia protein,

analogs and/or fragments, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second antibody may have an associated activity, such as an enzymatic activity, that will generate, for example, a color development, upon incubating with an appropriate chromogenic substrate. Quantification may then achieved by measuring the degree of color generation using, for example, a visible spectra spectrophotometer.

Use of Sequences as Hybridization Probes

The nucleotide sequences of the present invention, comprising the newly-isolated and characterized sequences of the *hia* genes, allow for the identification and cloning of the *hia* genes from other non-typeable strains of *Haemophilus*.

The nucleotide sequences comprising the sequence of *hia* genes of the present invention are useful for their ability to selectively form duplex molecules with complementary stretches of other *hia* genes. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity of the probe toward the other *hia* genes in other strains of non-typeable *Haemophilus*. For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt and/or high temperature conditions, such as

provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amount of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide and 0.15 M NaCl are: 42°C for an *hia* gene which is about 95 to 100% homologous to the target nucleic acid fragment, 37°C for about 90 to 95 homology and 32°C for about 85 to 90% homology.

In a clinical diagnostic embodiment, the nucleic acid sequences of the *hia* genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific

hybridization with samples containing *Hia* genes sequences.

The nucleic acid sequences of *Hia* genes of the present invention are useful as hybridization probes in solution hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-phase procedures the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e.g., serum, amniotic fluid, middle ear effusion, sputum, bronchoalveolar lavage fluid) or even tissues, is adsorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the *hia* genes or fragments thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. It is preferred to select nucleic acid sequence portions which are conserved among species of *Haemophilus*. The selected probe may be at least 18 bp in length and may be in the range of 30 bp to 90 bp long.

Expression of the *Haemophilus influenzae* adhesin Genes

Plasmid vectors containing replicon and control sequences which are derived from species compatible

with the host cell may be used for the expression of the *hia* genes in expression systems. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* may be transformed using pBR322 which contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage, must also contain, or be modified to contain, promoters which can be used by the host cell for expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEM™-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as *E. coli* LE392.

Promoters commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems and other microbial promoters, such as the T7 promoter system employed herein in preferred embodiments (U.S. Patent 4,952,496). Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with genes. The particular promoter used will generally be a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the Hia protein and immunological fragments or analogs thereof include *E.*

coli, *Bordetella* species, *Bacillus* species, *Haemophilus*, fungi, yeast or the baculovirus expression system may be used. *E. coli* is the preferred host used herein.

In accordance with this invention, it is preferred to produce the Hia proteins by recombinant methods, particularly when the naturally occurring Hia protein as purified from a culture of a species of *Haemophilus* may include trace amounts of toxic materials or other contaminants. This problem can be avoided by using recombinantly produced Hia protein in heterologous systems which can be isolated from the host in a manner to minimize contaminants in the purified materials, specifically employing the constructs described herein.

BIOLOGICAL DEPOSITS

A vector that contains nucleic acid coding for a high molecular weight protein of a non-typeable strain of *Haemophilus* that is described and referred to herein has been deposited with the America Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA, pursuant the Budapest Treaty and prior to the filing of this application. Samples of the deposited vector will become available to the public and all restrictions imposed or access to the deposits will be received upon grant of a patent based on this United States patent application. In addition, the deposit will be replaced if viable samples cannot be dispensed by the Depository. The invention described and claimed herein is not limited in scope by the biological materials deposited, since the deposited embodiment is intended

only as an illustration of the invention. Any equivalent or similar vectors that contain nucleic acid which encodes equivalent or similar antigens as described in this application are within the scope of the invention.

Deposit Summary

Plasmid	ATCC	Deposit Date
BK-96-2-11	203771	February 11, 1999

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, immunology and fermentation technology used, but not explicitly described in this disclosure and these Examples, are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example describes the construction of plasmid DS-2008-2-3 that expresses full-length rHia proteins from NTHi strain 11.

Chromosomal DNA was purified from NTHi strain 11 and the full-length *hia* gene was PCR amplified using the oligonucleotides (5038.SL and 5039.SL) described in Figure 1B. An Nde I site was engineered at the 5'-end of the gene and a BamH I site was engineered at the 3'-end for cloning into the pT7-7 expression vector (ref. 21). The amplified fragment was digested with Nde I/BamH I and cloned into pT7-7 that had been digested with the same enzymes. Plasmid DS-2008-2-3 contains a 3.4 kb strain 11 *hia* gene downstream of the T7 promoter (Fig. 1A). The plasmid was used to express recombinant Hia (Example 9 below).

Example 2

This Example illustrates the recognition of rHia by anti-native *Moraxella catarrhalis* high molecular weight adhesin antibody.

There is some sequence conservation observed between the *Haemophilus influenzae* Hia proteins and a *Moraxella catarrhalis* high molecular weight adhesin identified as the *M. catarrhalis* 200 kDa protein in aforementioned US Patent No. 5,808,024 (Fig. 28). The native *M. catarrhalis* 200 kDa protein was gel purified as described in US Patent No. 5,808,024 and guinea pig anti-native 200 kDa antibody was generated. The T7 *hia* gene was expressed from plasmid DS-2008-2-3 and the cell culture containing the rHia protein was electroblotted to nitrocellulose membrane. Immunoblot analysis using anti-native 200 kDa antibody showed that the antibody recognized the rHia protein, as seen in Figure 2.

Example 3

This Example describes the construction of plasmids DS-2092-1 and DS-2092-40 that contain tandem copies of *T7 hia* (11) gene cassettes.

In order to improve the production of full-length recombinant Hia protein, tandem copies of the *T7 hia* gene cassette containing the strain 11 *hia* gene (Example 1) were inserted into a single vector. Plasmid DS-2008-2-3 was linearized with *Bgl* II and dephosphorylated. Plasmid DS-2008-2-3 was also digested with *Bgl* II and *BamH* I to excise the *T7 hia* gene cassette. The *T7 hia* fragment was ligated into the linearized vector to generate plasmid DS-2092-1 that contains two copies of the *T7 hia* gene in the anti-clockwise orientation (a,a) and plasmid DS-2092-40 that contains tandem copies in opposite orientations (a,c) (Fig. 3). There was no obvious improvement in expression of rHia from either construct (see Example 9 below).

Example 4

This Example describes the construction of plasmids expressing truncated strain 11 *hia* genes.

The production of the rHia protein from single or tandem copies of the *T7 hia* gene cassette was very low and the protein seemed to be toxic to *E. coli* (as described below in Example 9). Since *H. influenzae* Hia is a surface-exposed adhesin molecule, it must either utilize a signal sequence or accessory protein(s) for secretion, but there are no known accessory genes involved. If the signal sequence were removed for expression of the recombinant protein in *E. coli*, the

rHia might be expressed as inclusion bodies and the toxic effect reduced. A putative signal sequence and cleavage sites were identified and four constructs expressing N-terminally truncated rHia proteins were designed (Fig. 4). There is a unique *Sty* I site in the strain 11 *hia* gene about 500 bp from the start codon. Plasmid DS-2008-2-3 was digested with *Nde* I and *Sty* I and the 5.7 kb vector fragment purified (Fig. 5A). PCR primers were designed to amplify from the truncation site to the *Sty* I site and a unique *Nhe* I site was introduced into the antisense primer for screening truncated clones (Fig. 5B). The amplified fragments were subcloned into pCRII for easier manipulation, generating plasmids DS-2153R-1-2 (E21), DS-2165-4-8 (T33), DS-2153-3-5 (V38), and DS-2153-4-4 (N52). The pCRII *hia* plasmids were digested with *Nde* I and *Sty* I and the fragments ligated with the vector piece from DS-2008-2-3. Plasmids DS-2186-1-1 (E21), DS-2201-1 (T33), DS-2186-2-1 (V38), and DS-2168-2-6 (N52) were generated that contained the T7 promoter and truncated *hia* genes as indicated in parentheses. These plasmids were used to express recombinant Hia (see Example 9 below).

Example 5

This Example describes the construction of plasmid BK-96-2-11 that contains the *T7 V38 hia (11)* cassette, the *E. coli cer* gene, and the kanamycin resistance gene.

Plasmid DS-1843-2 is a pBR328-based plasmid in which a multiple cloning site and two transcription terminators have been introduced on oligonucleotides,

between the *EcoR* I and *Pst* I sites, thus destroying both the chloramphenicol and ampicillin resistance genes (Fig. 6B). The kanamycin resistance gene from pUC-4K was inserted at the *Sal* I site, to generate plasmid DS-2147-1 that is kanamycin resistant and tetracycline sensitive. Plasmid DS-2224-1-4 is a pUC plasmid containing a synthetic *E. coli cer* gene (ref. 15) constructed from oligonucleotides and flanked by *BamH* I sites. The 290 bp *BamH* I fragment of the *cer* gene was inserted into the *BamH* I site of DS-2147-1 creating plasmid BK-2-1-2. This pBR-based plasmid thus contains a multiple cloning site, the kanamycin resistance gene and the *cer* gene. Plasmid BK-2-1-2 was linearized with *Bgl* II and dephosphorylated. Plasmid DS-2186-2-1 was digested with *Bgl* II and *BamH* I and the 3.6 kb *T7 V38 hia* fragment was inserted into BK-2-1-2, creating plasmid BK-96-2-11 (Fig. 6A).

Example 6

This Example describes the construction of plasmids DS-2242-1 and DS-2242-2 that express the full-length NTHi strain 33 *hia* gene in the presence of the *E. coli cer* gene.

Chromosomal DNA was purified from NTHi strain 33 and PCR amplification was performed using oligonucleotides 5039.SL and 5040.SL (Fig. 17). The sense primer (5040.SL) was designed based upon the 5'-flanking sequence of strain 11 *hia* and the conserved amino terminal sequences of the NTHi Hia and Hib Hsf proteins. The antisense primer (5039.SL) was the same as that described in Example 1 and was based upon the conserved carboxy terminal sequences of the Hia and Hsf

proteins. The 3 kb strain 33 *hia* PCR fragment was cloned into pCR II, generating plasmid DS-1917-3-8.

In order to express the full-length strain 33 *hia* gene, approximately 106 bp of the 5'-end of the gene was synthesized from oligonucleotides, from the start codon to an *AlwN* I site (Fig. 7B). Plasmid DS-1917-3-8 was digested with *AlwN* I and *BamH* I and the approximately 2.9 kb fragment containing the *hia* gene was purified. Plasmid pT7-7 was digested with *Nde* I and *BamH* I. The *Nde* I - *AlwN* I oligonucleotides and *AlwN* I - *BamH* I *hia* fragment were ligated into the pT7-7 vector, generating plasmid DS-2103-4.

In order to include the *E. coli cer* gene and utilize kanamycin selection, the *Bgl* II - *BamH* I fragment containing the *T7 hia (33)* gene cassette was excised from DS-2103-4 and cloned into BK-2-1-1 that had been digested with *Bgl* II and dephosphorylated. Plasmids DS-2242-1 and DS-2242-2 contain single copies of the *T7 hia (33)* gene cassette in opposite orientations, the *E. coli cer* gene, and the kanamycin resistance gene (Fig. 7A).

Example 7

This Example describes the construction of plasmid DS-2340-2-3 that contains a *T7 hia* gene cassette with a truncated V38 strain 33 *hia* gene, the *E. coli cer* gene, and the kanamycin resistance gene.

PCR primers were designed to amplify a 250 bp fragment of the 5'-end of the NTHi strain 33 hia gene from a V38 start codon up to an internal *SnaB* I site. An *Nde* I site was added at the 5'-end for cloning purposes and the fragment was amplified using plasmid

DS-2242-1 as template. The construction scheme is shown in Figure 8A and the PCR primers are shown in Figure 8B. The fragment was cloned into pCR II generating plasmid DS-2328-1-1. DS-2242-1 was digested with *Nde* I and *SnaB* I and the 8.5 kb vector fragment purified. DS-2328-1-1 was digested with *Nde* I and *SnaB* I and the 0.25 kb *5' hia* fragment was ligated with the 8.5 kb vector fragment from DS-2242-1, to generate plasmid DS-2340-2-3.

Example 8

This Example illustrates the construction of plasmids DS-2447-2 and DS-2448-17 that contain tandem copies of *T7 V38 hia (11)* or *T7 V38 hia (33)* gene cassettes, respectively, the *E. coli cer* gene, and a kanamycin resistance gene.

Plasmid BK-96-2-11, that contains a *T7 V38 hia (11)* gene cassette, was linearized with *Bgl* II and dephosphorylated. The *Bgl* II-*BamH* I *T7 V38 hia (11)* gene cassette from DS-2186-2-1 was ligated into BK-96-2-11, generating plasmid DS-2447-2 that contains tandem copies of the *T7 V38 hia (11)* gene in the same orientation (Fig. 9A).

Plasmid DS-2340-2-3 was digested with *EcoR* I and the *T7 V38 hia (33)* gene cassette was subcloned into pUC-BgXb that had been digested with *EcoR* I and dephosphorylated. The resultant plasmid, DS-2440-2 was digested with *Bgl* II and *BamH* I to release the *T7 V38 hia (33)* cassette that was ligated with DS-2340-2-3 that had been linearized with *Bgl* II and dephosphorylated. Plasmid DS-2448-17 contains tandem *T7 V38 hia(33)* genes in the same orientation (Fig. 9B).

Example 9

This Example illustrates the expression of full-length and truncated recombinant *hia* genes.

DNA from expression plasmids prepared as described in the preceding Examples, was introduced into electrocompetent *E. coli* BL21 (DE3) cells using a BioRad electroporator. Cells were grown at 37°C in NZCYM medium using the appropriate antibiotic selection to A_{578} of 0.3 before the addition of lactose to 1.0% for 4 hours. Samples were adjusted to 0.2 OD/µl with SDS-PAGE lysis + loading buffer and the same amount of each protein sample was loaded onto SDS-PAGE gels (ref. 22). Figure 10 illustrates the relative production of rHia (11) proteins from various constructs. As seen in panel A, there is an increase in production with decreased size of rHia. V38- (lane 5) and N52-truncated rHia (lane 6) have significantly higher expression levels than their longer counterparts (lanes 2, 3, 4). In addition, panel B demonstrates that the production of V38 rHia is apparently increased in the presence of the *cer* gene.

Example 10

This Example illustrates the purification of rHia proteins.

All the recombinant Hia proteins were expressed as inclusion bodies in *E. coli* and were purified by the same procedure (Fig.11). *E. coli* cell pellets from 500 ml culture were resuspended in 50 ml of 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl, and disrupted by sonication. The extract was centrifuged at 20,000 *g* for 30 min and the resultant supernatant was discarded.

The pellet (PPT_1) was further extracted, in 50 ml of 50 mM Tris-HCl, pH 8.0 containing 0.5% Triton X-100 and 10 mM EDTA, then centrifuged at 20,000 *g* for 30 min, and the supernatant was discarded. The pellet (PPT_2) was further extracted in 50 ml of 50 mM Tris-HCl, pH 8.0, containing 1% octylglucoside, then centrifuged at 20,000 *g* for 30 min, and the supernatant was discarded.

The resultant pellet (PPT_3) obtained after the above extractions contains the inclusion bodies. The pellet was solubilized in 6 ml of 50 mM Tris-HCl, pH 8.0, containing 6 M guanidine and 5 mM DTT. Twelve ml of 50 mM Tris-HCl, pH 8.0 was added to this solution and the mixture was centrifuged at 20,000 *g* for 30 min. The supernatant (SUP_4) was precipitated with polyethylene glycol (PEG) 4000 at a final concentration of 7%. The resultant pellet (PPT_5) was removed by centrifugation at 20,000 *g* for 30 min and the supernatant was precipitated by $(NH_4)_2SO_4$ at 50% saturation. The $(NH_4)_2SO_4$ precipitate was collected by centrifugation at 20,000 *g* for 30 min. The resultant pellet (PPT_6) was dissolved in 2 ml of 50 mM Tris-HCl, pH 8.0, containing 6 M guanidine HCl and 5 mM DTT and the clear solution was purified on a Superdex 200 gel filtration column equilibrated in 50 mM Tris-HCl, pH 8.0, containing 2 M guanidine HCl. The fractions were analysed by SDS-PAGE and those containing the purified rHia were pooled and dialysed overnight at 4°C against PBS, then centrifuged at 20,000 *g* for 30 min. The protein remained soluble under these conditions and glycerol was added to the rHia preparation at a final concentration of 20% for storage at -20°C. SDS-PAGE

analysis of purified V38 rHia (11) and V38 rHia (33) is illustrated in Figure 12. The average yield of the purified V38 rHia proteins is about 10 mg L^{-1} culture.

In order to study the stability of rHia, the purified V38 rHia (11) protein was stored at 4°C with or without glycerol and at -20°C with glycerol. The protein was found to be stable under all three conditions and remained intact for at least eight weeks with repeated freezing and thawing (Fig. 13).

Example 11

This Example illustrates the immunogenicity of V38 rHia (11) and V38 rHia (33) proteins.

Hyperimmune antisera against rHia proteins were produced by immunizing two guinea pigs (Charles River) intramuscularly (i.m.) with 5 µg doses of antigen emulsified in complete Freund's adjuvant (CFA, Difco) on day 1. Animals were boosted on days 14 and 28 with 5 µg doses of protein in incomplete Freund's adjuvant (IFA) and sera were collected on day 42. Anti-Hib strain MinnA and anti- *Haemophilus* type a strain ATCC 9006 antisera were generated using the same protocol, except that a heat-inactivated bacterial preparation was used as the immunogen ($1x10^8$ cfu per dose).

To study the immunogenicity of the V38 rHia proteins, groups of five CD-1 mice (Charles River, Quebec) were immunized s.c. on days 1 and 28 with 0.3, 1, 3, and 10 µg of antigen, in the presence of $AlPO_4$ (alum) (1.5 mg per dose). Blood samples were collected on days 1, 28 and 42. Mice generated significant anti-V38 rHia antibody responses even with a single injection of 0.3 µg antigen (Fig. 14, panel A),

suggesting that both proteins had retained immunogenicity after inclusion body extraction and solubilization. No statistically significant difference was found in the antibody titers induced by the V38 rHia proteins derived from strains 11 or 33.

To study the immunogenicity of the V38 rHia (11) protein in BALB/c mice, groups of five animals (Charles River, Quebec) were immunized s.c. on days 1, 28 and 42 with 0.3, 1, 3, and 10 µg of antigen, in the presence of $AlPO_4$ (1.5 mg per dose). Blood samples were collected on days 1, 14, 28, 42 and 56. High antibody titers were observed in all groups, indicating that the protein is very immunogenic even at 0.3 µg per dose (Fig. 15, panel A).

To study the immunogenicity of the V38 rHia (11) protein in guinea pigs, groups of five animals (Charles River, Quebec) were immunized s.c. on days 1, 28 and 42 with 0.3, 1, 3, and 10 µg of antigen, in the presence of $AlPO_4$ (1.5 mg per dose). Blood samples were collected on days 1, 14, 28, 42 and 56. High antibody titers were observed in all groups, indicating that the protein is also very immunogenic in guinea pigs (Fig. 15, panel B).

Example 12

This Example illustrates the analysis of the protection afforded by anti-rHia antibodies in passive infant rat models of bacteremia.

Pregnant Wistar rats were purchased from Charles River. In the *H. influenzae* type b bacteremia model, groups of 6 to 10 five-day old infant rats were injected s.c. in the dorsal region with 0.1 ml of

guinea pig anti-rHia or anti-strain MinnA antiserum. The control animals received injections with pre-immune sera only. Twenty hours later, the animals were challenged intraperitoneally (i.p.) with 200 to 240 colony-forming units (cfu) of freshly grown Hib strain MinnA (0.1 ml). Blood samples were collected 20 h post-challenge, via cardiac puncture under isoflurane anesthesia and plated on chocolate agar plates. Colonies were counted after one day and the results were statistically analyzed by Fisher's Exact test.

In the *H. influenzae* type a bacteremia model (ref. 23), groups of 9 to 10 five-day old infant rats were injected s.c. in the dorsal region with 0.1 ml of guinea pig anti-rHia or anti-strain ATCC 9006 antiserum. The animals in the control group were injected with guinea pig pre-immune serum. Twenty hours later, the animals were challenged i.p. with 100,000 cfu of freshly grown *H. influenzae* type a strain ATCC 9006 (0.1 ml). Blood samples were collected 20 h post-challenge and analysed as described above.

As shown in Tables 1 and 2 below, the infant rats that were passively immunized with either guinea pig anti-rHia (11) or anti-V38 rHia (11) antisera, were all significantly protected against type a or type b *H. influenzae* caused bacteremia. These results demonstrate that antibodies raised to the slightly truncated Hia protein (V38 rHia) are as efficacious as those raised to the full-length protein at protecting animals against bacteremia caused by type a or type b *H. influenzae*. Such protection afforded by an NTHi-derived recombinant protein against invasive disease

caused by encapsulated bacteria, illustrates the utility of the rHia proteins as vaccine antigens.

Example 13

This Example illustrates the protection afforded by immunization with V38 rHia protein in a chinchilla model of nasopharyngeal colonization.

A nasopharyngeal colonization model has been described by Yang et al (ref. 20). The model works well for those NTHi strains that produce the HMW adhesins, but reproducible colonization could not be established with Hia-producing strains under the same conditions. Repeated attempts to colonize with the prototype Hia-producing NTHi strain 11, were unsuccessful. Colonization was achieved with NTHi strain 33 at 5 x 10^8 cfu per inoculum, compared with only 10^8 cfu required for the prototype HMW-producing NTHi strain 12. Under these conditions, partial protection was observed in animals immunized with 100 µg of V38 rHia (33) and challenged with the homologous NTHi strain 33.

Example 14

This Example illustrates the cloning and sequence analysis of additional *hia* genes from *H. influenzae* strains.

Oligonucleotides (5040.SL and 5039.SL) for PCR amplification were designed based upon the conserved promoter, N-terminal and C-terminal sequences of the *hia* and *hsf* genes and proteins (Fig. 17). The strains chosen for PCR amplification were chosen based upon their reactivity with anti-rHia (11) antisera.

Chromosomal DNA was prepared from NTHi strains 12, 29, 32, M4071, K9 and, K22 and *Haemophilus* type c strain API. PCR amplification was performed as follows: each reaction mixture contained 5 to 100 ng of DNA, 1 μg of each primer, 5 units of taq+ or tsg+ (Sangon) or taq plus long (Stratagene), 2 mM dNTPs, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100, BSA. Cycling conditions were: 95°C for 1 min, followed by 25 cycles of 95°C for 30 sec, 45°C for 1 min, 72°C for 2 min; then 72°C for 10 min.

The nucleotide and deduced amino acid sequences of the *hia* gene from strain 33 are shown in Figure 18. The predicted Hia protein from strain 33 has a molecular weight of 103.6 kDa and a pI of 9.47. The nucleotide and deduced amino acid sequences of the *hia* gene from strain 32 are shown in Figure 19. The predicted Hia protein from strain 32 has a molecular weight of 70.4 kDa and a pI of 5.67. There is a KDEL sequence present between residues 493 and 496. Such sequences have been associated with anchoring proteins to the endoplasmic reticulum. The deduced strain 32 Hia protein is significantly smaller and has a significantly different pI, however it does contain many of the motifs present in other Hia molecules.

The nucleotide and deduced amino acid sequences of the *hia* gene from strain 29 are shown in Figure 20. The predicted Hia protein from strain 29 has a molecular weight of 114.4 kDa and a pI of 7.58. The nucleotide and deduced amino acid sequences of the *hia* gene from strain K22 are shown in Figure 23. The predicted Hia protein from strain K22 has a molecular weight of 114.4

kDa and a pI of 7.58. The deduced Hia sequences from NTHi strains 29 and K22 were found to be identical. Strain 29 was isolated from a 7-month old child with otitis media in Cleveland, Ohio, while strain K22 was isolated from an aborigine near Kimberly, Australia.

The nucleotide and deduced amino acid sequences of the *hia* gene from strain 4071 are shown in Figure 21. The predicted Hia protein from strain M4071 has a molecular weight of 103.4 kDa and a pI of 9.49. There is a KDEL sequence present between residues 534 and 537.

The nucleotide and deduced amino acid sequences of the *hia* gene from strain K9 are shown in Figure 22. The predicted Hia protein from K9 has a molecular weight of 113.8 kDa and a pI of 6.45.

The nucleotide and deduced amino acid sequences of the *hia* gene from strain type c *Haemophilus* API are shown in Figure 24. The predicted Hia protein from API has a molecular weight of 249.4 kDa and a pI of 5.34. The deduced Hia/Hsf sequence from the type c strain API is nearly identical to the published type b Hsf sequence except for a 60 residue insert. Since the NTHi-based Hia protein provided herein protects in passive models of type a and type b infection, it is likely that it will also protect against type c disease due to sequence similarity between the type b and type c proteins.

The nucleotide and deduced amino acid sequences of the *hia* locus from strain 12 are shown in Figure 25. NTHi strain 12 does not produce Hia. However, part of the *hia* gene can be PCR amplified, there is

inconsistent positive reactivity of SB12 cell lysates with anti-rHia antibody, and there is reactivity with a DNA probe derived from the 3'-end of the strain 11 *hia* gene, on Southern blots. Analysis of the PCR amplified DNA, revealed a 1.8 kb fragment that contains 1 kb of the 3'-end of the upstream HI1732-related gene and 0.8 kb of the 3'-end of the *hia* gene.

PCR amplification using primers that would amplify across the putative junction of these two genes in strain 12, confirmed the genetic composition of the locus. Thus it would appear that strain 12 does not produce Hia because it has suffered a deletion of the 5'-end of the *hia* gene. Figure 27 shows a sequence comparison between the upstream orf of strain 12 and the Rd genome deduced HI1733 protein. Over the region of homology, the two proteins are 95% identical.

An alignment of the deduced Hia sequences from NTHi strains 33, 32, 29, K22, M4071, 11 and K9 and type c strain API compared with *H. influenzae* type b Hsf, the aidA-like (Hsf/Hia) HI1732 gene from the Rd genome, and the *M. catarrhalis* 200 kDa protein from strains 4223 and LES-1 is shown in Figure 28. There is a frame shift in the Rd genome sequence resulting in premature truncation of the HI1732 protein. Additional downstream sequence related to hia, is included here. The asterisks below the sequence indicate conserved residues. The N-terminal (approximately 50 residues) and C-terminal sequences (approximately 150 residues) are highly conserved amongst the *Haemophilus* strains, while some similarity is evident with the *M. catarrhalis* counterpart. Sequence analysis reveals that

there are two potential gene families of Hia proteins, one related to the prototype strain 11 and the other more closely related to strain 33. The strains 11 and K9 proteins appear to be more like the Hsf proteins from the type b, type c or type d *Haemophilus* strains while the strains 33, 32, 29, K22 and M4071 proteins appear to form a second family.

Example 15

This Example describes the construction of plasmid JB-2930-3 that contains a *T7 hia* gene cassette with a truncated S44 strain 11 *hia* gene, the *E. coli cer* gene, and the kanamycin antibiotic resistance gene, and expression of S44 Hia proteins.

PCR primers were designed to amplify the S44 Hia N-terminus of the NTHi strain 11 *hia* gene from the S44 amino acid to an internal *Sty* I site (Fig 29). An *Nde* I site was added at the 5'-end for cloning purposes and the fragment was amplified using plasmid DS-2242-1 as a template. The fragment was cloned into pCR II generating plasmid JB-2910-1-1. The construction scheme is shown in Figure 30. Plasmid JB-2910-1-1 was digested with *Nde* I and Sty I and the 5' PCR *hia* fragment isolated. Plasmid IA-46-5 containing the V38 hia gene was digested with *Nde* I and Sty I and the larger approximately 8.5 kb fragment purified. The two purified fragments were ligated together to produce plasmid JB-2917-1. This plasmid was then digested with *Nde* I and treated with calf intestinal phosphatase (CAP), and into it was cloned the T7 promoter from plasmid IA-46-5. The promoter was cut out using *Nde* I digestion of IA-46-5. The resulting plasmid, JB-2925-3,

was digested with *Bgl* II and *Bam* HI and the *hia* gene was isolated. This fragment was ligated into the *Bgl* II/CAP-treated plasmid BK-2-1-2 to produce plasmid JB-2930-3. This plasmid contains the T7 promoter S44 *hia* gene and *E. coli cer* gene and kanamycin resistance.

The recombinant S44 *hia* vector was transformed into *E. coli* BL21(DE3) for expression studies. The procedure for expression in *E. coli* was as described in Example 9. Figure 31 SDS-PAGE analysis of shows the expression of recombinant S44 *hia* from two different vectors, JB-2930-3 (described above) and pET vector IA-191-3-1. Plasmid IA-191-3-1 is identical to JB-2930-3 except it is a pET vector containing the $lacI^q$ repressor and, therefore, the amount of S44 *Hia* produced is less than the T7 S44 from JB-2930-3. The plasmid is shown, along with plasmid JB-2930-3, Figure 32. Figure 31 shows the S44 *Hia* as a doublet band (lane 3) at approximately 116 kDa. Upon further analysis using purified S44 *hia* from JB-2930-3, the lower band of the doublet was found to have a C-terminal truncation of 94 amino acids, while retaining the expected N-terminus. The purification process used for isolation of the truncated Hia was as described in Example 10.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides novel isolated and purified nucleic acid molecules encoding full-length and N-terminal truncated *Haemophilus influenzae* adhesin (Hia) proteins from *Haemophilus* which enable protective Hia proteins

to be produced recombinantly. Modifications are possible within the scope of this invention.

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TABLE 1

Protective effect of guinea pig anti-rHia (full-length) antiserum against type a or b *H. influenzae* in the infant rat model of bacteremia

Group (#)	Guinea pig serum	Anti-rHia antibody titers	No. bacteremic/ No. challenged	Mean cfu/ 100 µl blood
1	Anti-type a	nd	0/10*	0**
2	Anti-rHia	204,800	1/10*	0**
3	Preimmune	<100	7/10	88
Group (#)	Guinea pig serum	anti-rHia antibody titers	No. bacteremic/ No. challenged	Mean cfu/ 2.5 µl blood
4	Anti-MinnA	nd	0/10*	0**
5	Anti-rHia	204,800	1/10*	2**
6	Preimmune	<100	10/10	600

Five-day old infant rats were passively immunized s.c. with 0.1 ml of indicated guinea pig antiserum or preimmune serum. Twenty hours later, infant rats were challenged i.p. with either freshly grown *H. influenzae* type a strain ATCC 9006 (10^5 cfu, 0.1 ml) for groups #1 to 3; or with freshly grown Hib strain MinnA (240 cfu, 0.1 ml) for groups # 4 to 6. Infected animals are defined as >20 cfu recovered from 100 µl of blood for groups #1 to 3; or >30 cfu recovered from 2.5 µl of blood for groups # 4 to 6.

* Fisher exact test. Statistical significance compared to animals in group 3 or 6 was found ($P<0.05$).

** Student's unpaired *t* test. Statistical significance compared to animals in group 3 or 6 was found ($P<0.05$).

nd: not determined.

TABLE 2

Protective effect of guinea pig anti-V38 rHia (SB11) antiserum against type a or b *H. influenzae* in the infant rat model of bacteremia

Group (#)	Guinea pig serum	Anti-rHia antibody titers	No. bacteremic/ No. challenged	Mean cfu/ 20 µl blood
1	Anti-type a	nd	0/6*	0**
2	Anti-rHia	204,800	1/9*	5**
3	Preimmune	<100	5/8	165
Group (#)	Guinea pig serum	anti-rHia antibody titers	No. bacteremic/ No. challenged	Mean cfu/ 2 µl blood
4	Anti-MinnA	nd	0/6*	0**
5	Anti-rHia	204,800	1/9*	2**
6	Preimmune	<100	10/10	820

Five-day old infant rats were passively immunized s.c. with 0.1 ml of indicated guinea pig antiserum or preimmune serum. Twenty hours later, infant rats were challenged i.p. with either freshly grown *H. influenzae* type a strain ATCC 9006 (10^5 cfu, 0.1 ml) for groups #1 to 3; or with freshly grown Hib strain MinnA (190 cfu, 0.1 ml) for groups #4 to 6. Infected animals is defined as >20 cfu recovered from 20 µl of blood for groups #1 to 3; or >30 cfu recovered from 2 µl of blood for groups #4 to 6.

* Fisher exact test. Statistical significance compared to animals in group 3 or 6 was found ($P<0.05$)

** Student's unpaired *t* test. Statistical significance compared to animals in group 3 or 6 was found ($P<0.05$).

nd: Not determined.

CLAIMS

What we claim is:

1. An isolated and purified nucleic acid molecule encoding a *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* having:

(a) a DNA sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 23, 25, 27, 29, 31, 33, 35, 37); or

(b) a DNA sequence encoding a *Haemophilus influenzae* adhesin (Hia) protein having an amino acid sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 24, 26, 28, 30, 32, 34, 36, 38).

2. An isolated and purified nucleic acid molecule encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* which is amplifiable by a pair of nucleotides which are selected from the group consisting of:

SEQ ID No: 7 and SEQ ID No: 15

SEQ ID No: 9 and SEQ ID No: 15

SEQ ID No: 11 and SEQ ID No: 15

SEQ ID No: 13 and SEQ ID No: 15

SEQ ID No: 49 and SEQ ID No: 51

3. An isolated and purified nucleic acid encoding a truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* expressible as inclusion bodies.

4. A vector for transforming a host comprising the nucleic acid molecule of claim 1.

5. A vector for transforming a host comprising the nucleic acid molecule of claim 2 or 3.

6. The vector of claim 4 or 5 which is a plasmid vector.

7. The vector of claim 6 wherein said plasmid vector has the identifying characteristics of a plasmid which is selected from the group consisting of:

DS-2008-2-3 as shown in Figure 1A

DS-2186-1-1 as shown in Figure 5A

DS-2201-1 as shown in Figure 5A

DS-2186-2-1 as shown in Figure 5A

DS-2168-2-6 as shown in Figure 5A

IA-191-3-1 as shown in Figure 32

8. A vector for transforming a host, comprising a nucleic acid molecule encoding a full-length or N-truncated *Haemophilus influenzae* adhesin (Hia) protein and a promoter for expression of said full-length or truncated Hia protein.

9. The vector of claim 8 further comprising the *cer* gene of *E. coli*.

10. The vector of claim 8 which is a plasmid vector.

11. The vector of claim 10 wherein said plasmid vector has the identifying characteristics of a plasmid vector which is selected from the group consisting of:

BK-96-2-11 as shown in Figure 6A

DS-2242-1 as shown in Figure 7A

DS-2242-2 as shown in Figure 7A

DS-2340-2-3 as shown in Figure 8A

DS-2447-2 as shown in Figure 9A

DS-2448-17 as shown in Figure 9B

JB-2930-3 as shown in Figure 32

12. A host cell transformed by a vector as claimed in claim 4, 5 or 8 and expressing a protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus*.

13. The host cell of claim 12 which is a strain of *E. coli*.

14. A recombinant protective *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* producible by the transformed *E. coli* of claim 13 or an immunogenic fragment or an adhesin-functional analog thereof.

15. An immunogenic composition, comprising at least one immunologically-active component selected from the group consisting of:

(A) an isolated and purified nucleic acid molecule encoding a *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* having:

(a) a DNA sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 23, 25, 27, 29, 31, 33, 35, 37); or

(b) a DNA sequence encoding a *Haemophilus influenzae* adhesin (Hia) protein having an amino acid sequence selected from the group consisting of those shown in Figures 18, 19, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 24, 26, 28, 30, 32, 34, 36, 38);

(B) an isolated and purified nucleic acid molecule encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* which is amplifiable by a pair of

nucleotides which are selected from the group consisting of:

SEQ ID No: 7 and SEQ ID No: 15

SEQ ID No: 9 and SEQ ID No: 15

SEQ ID No: 11 and SEQ ID No: 15

SEQ ID No: 13 and SEQ ID No: 15

SEQ ID No: 49 and SEQ ID No: 51;

(C) an isolated and purified nucleic acid molecule encoding a truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* expressible as inclusion bodies; and

(D) a recombinant protective *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* producible by a strain of *E. coli* transformed by an expression vector as claimed in claim 4, 5 or 8; and

a pharmaceutically-acceptable carrier therefor.

16. The immunogenic composition of claim 15 formulated as a vaccine for *in vivo* administration to protect against disease caused by *Haemophilus*.

17. The immunogenic composition of claim 15 in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces.

18. The immunogenic composition of claim 15 formulated as a microparticle, capsule or liposome preparation.

19. The immunogenic composition of claim 15 further comprising an adjuvant.

20. A method for inducing protection against disease caused by *Haemophilus*, comprising administering to a

susceptible host an effective amount of the immunogenic composition of claim 15.

21. The method of claim 20 wherein the susceptible host is a human.

22. A method for the production of a protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus influenzae*, which comprises:

transforming a host with a vector as claimed in claim 5,

growing the host cell to express the encoded truncated Hia, and

isolating and purifying the expressed Hia protein.

23. The method of claim 22 wherein the host cell is *E. coli*.

24. The method of claim 22 wherein said encoded truncated Hia is expressed in inclusion bodies.

25. The method of claim 24 wherein said isolation and purification of the expressed Hia is effected by:

disrupting the grown transformed cells to produce a supernatant and the inclusion bodies,

solubilizing the inclusion bodies to produce a solution of the recombinant Hia,

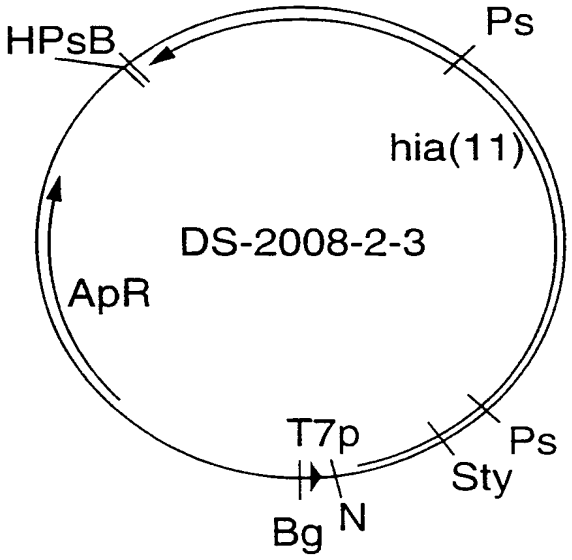
chromatographically purifying the solution of recombinant Hia free from cell debris, and

isolating the purified recombinant Hia protein.

26. The method of claim 22 wherein said non-typeable strain of *Haemophilus* is selected from the group consisting of strains 11, 33, 32, 29, M4071, K9, K22 and 12.

27. The method of claim 22 wherein said vector includes the T7 promoter and said *E. coli* is cultured in the presence of an inducing amount of lactose.

Restriction map of DS-2008-2-3, pT7 hia (11).



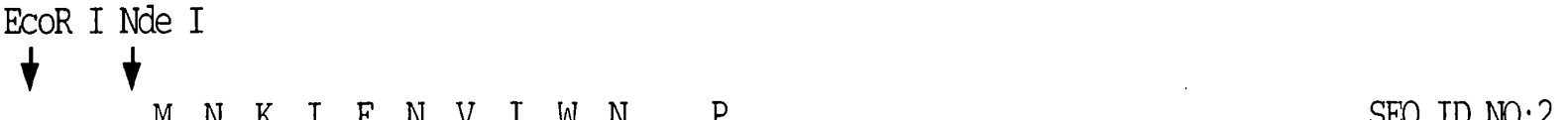
pT7 hia (11)

FIG.1A

FIG.1B

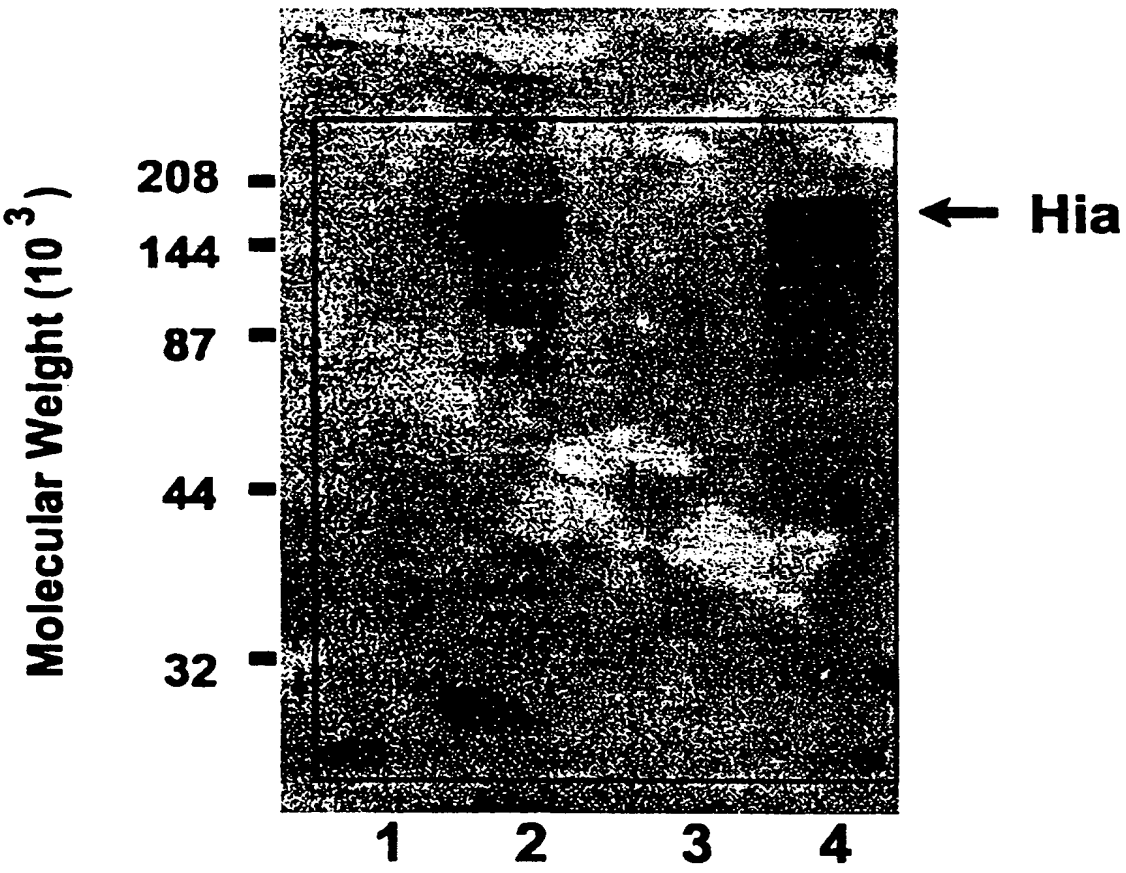
Oligonucleotides used to PCR amplify the full-length strain 11 *hia* gene for expression studies.

sense



antisense

FIG.2



Construction of DS-2092-1 and DS-2092-40, plasmids containing tandem T7 hia (11) genes.

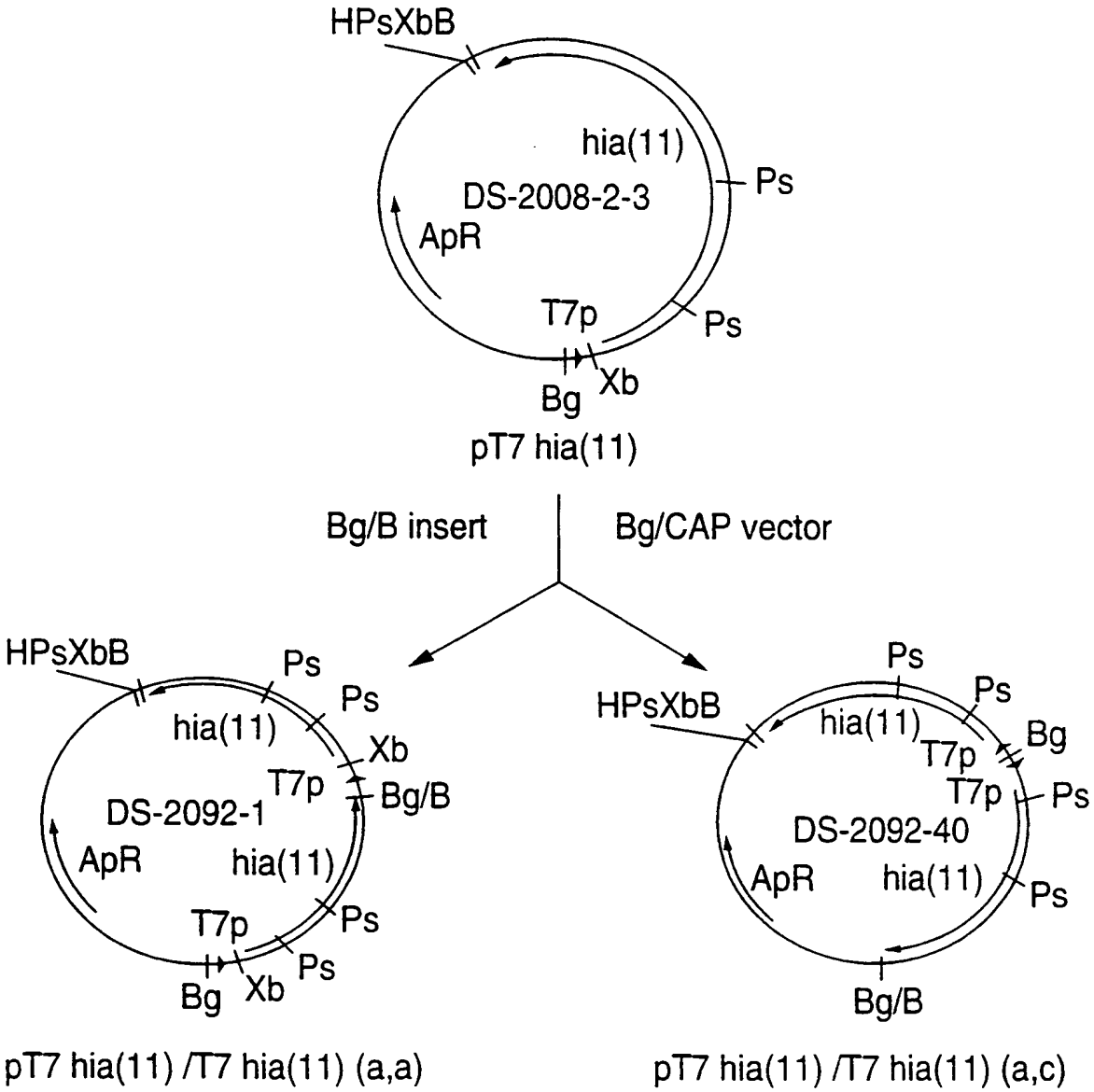


FIG.3

FIG.4

Sites for N-terminal truncations of rHia proteins.

(SEQ ID NO.6)

Construction of plasmids expressing truncated hia (11) genes.

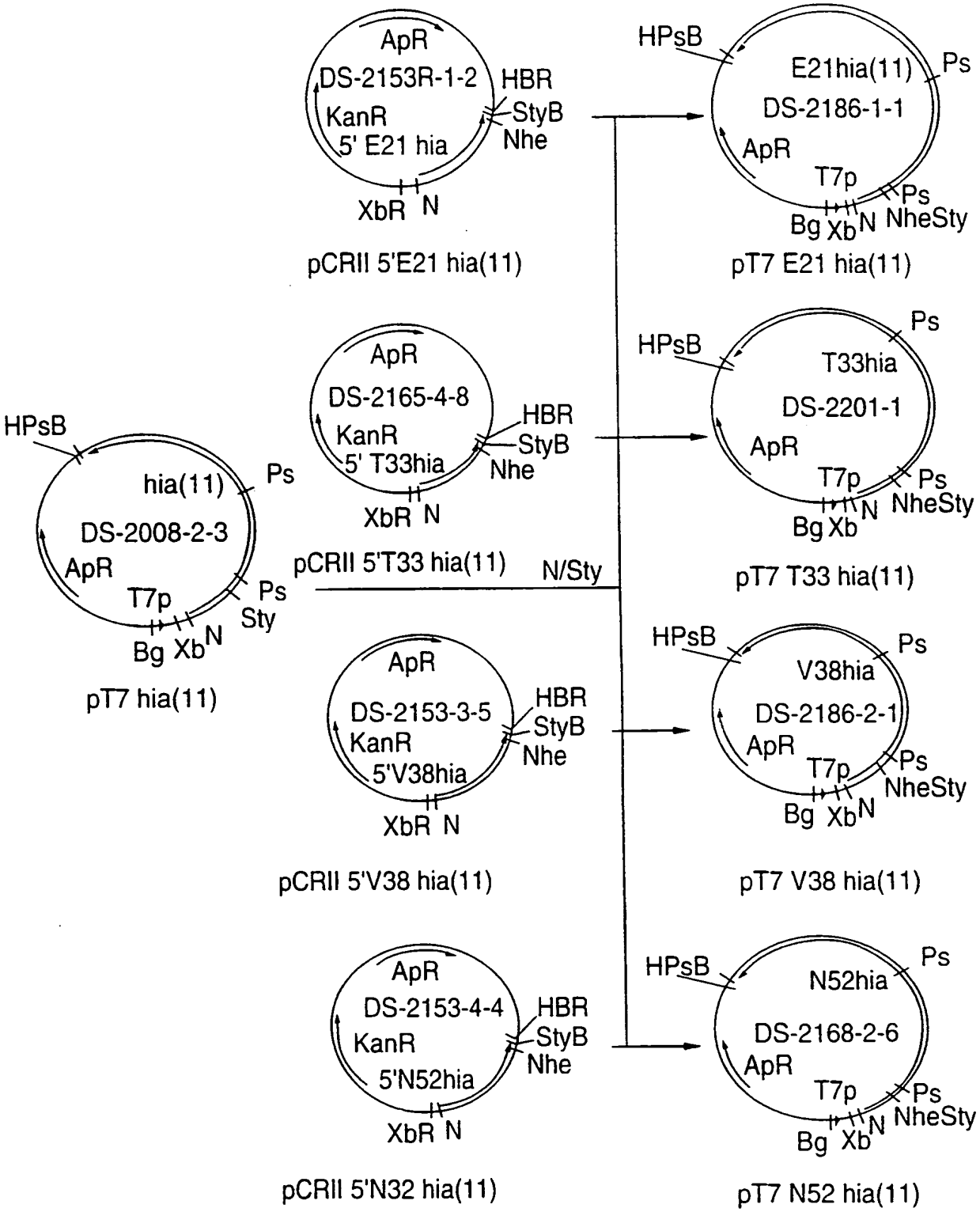


FIG.5A

FIG.5B

Oligonucleotide primers to PCR amplify truncated strain 11 *hia* genes.

E21

EcoR I Nde I

M E L T R T H T K C A

				SEQ ID NO: 8
5′	GGGAATTCATATGGAACTCACTCGCACCCACACCAAATGGGCC	3′	5524.SL	SEQ ID NO: 7

T33

M T V A V A V L A T L

				SEQ ID NO:10
5′	GGGAATTCATATGACCGTGGCGGTTGCCGTATTGGCAACCCTG	3′	5525.SL	SEQ ID NO: 9

V38

M V L A T L L S A T

				SEQ ID NO:12
5′	GGGAATTCATATGGTATTGGCAACCCTGTTGTCCGCAACG	3′	5526.SL	SEQ ID NO:11

FIG.5B'

N52

	M N T P V T N K L K A			SEQ ID NO:14
5′	GGGAATTCATATGAATACTCCTGTTACGAATAAGTTGAAGGCT	3′	5527.SL	SEQ ID NO:13

antisense

	H T I T F A L A K D L G			SEQ ID NO:17
5′	CACACCATTACCTTTGCGCTAGCGAAAGACCTTGGTGG	3′		SEQ ID NO:16
3′	GTGTGGTAATGGAAACGCGATCGCTTTCTGGAACCACCCTAGGGC	5′	5528.SL	SEQ ID NO:15

Nhe I Sty I BamH I

Construction of BK-96-2-11,
a plasmid containing T7 V38 hia(11) and cer.

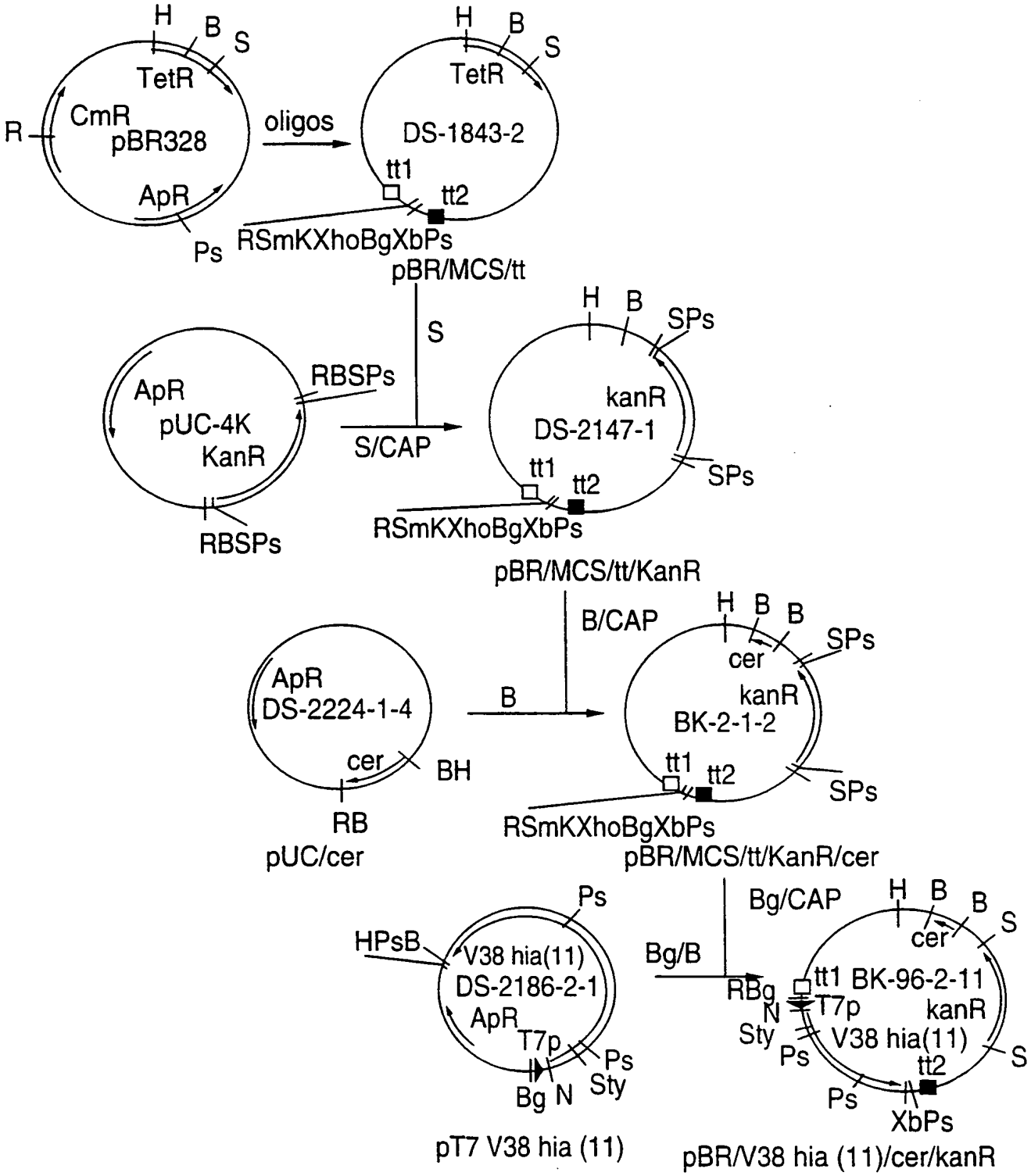
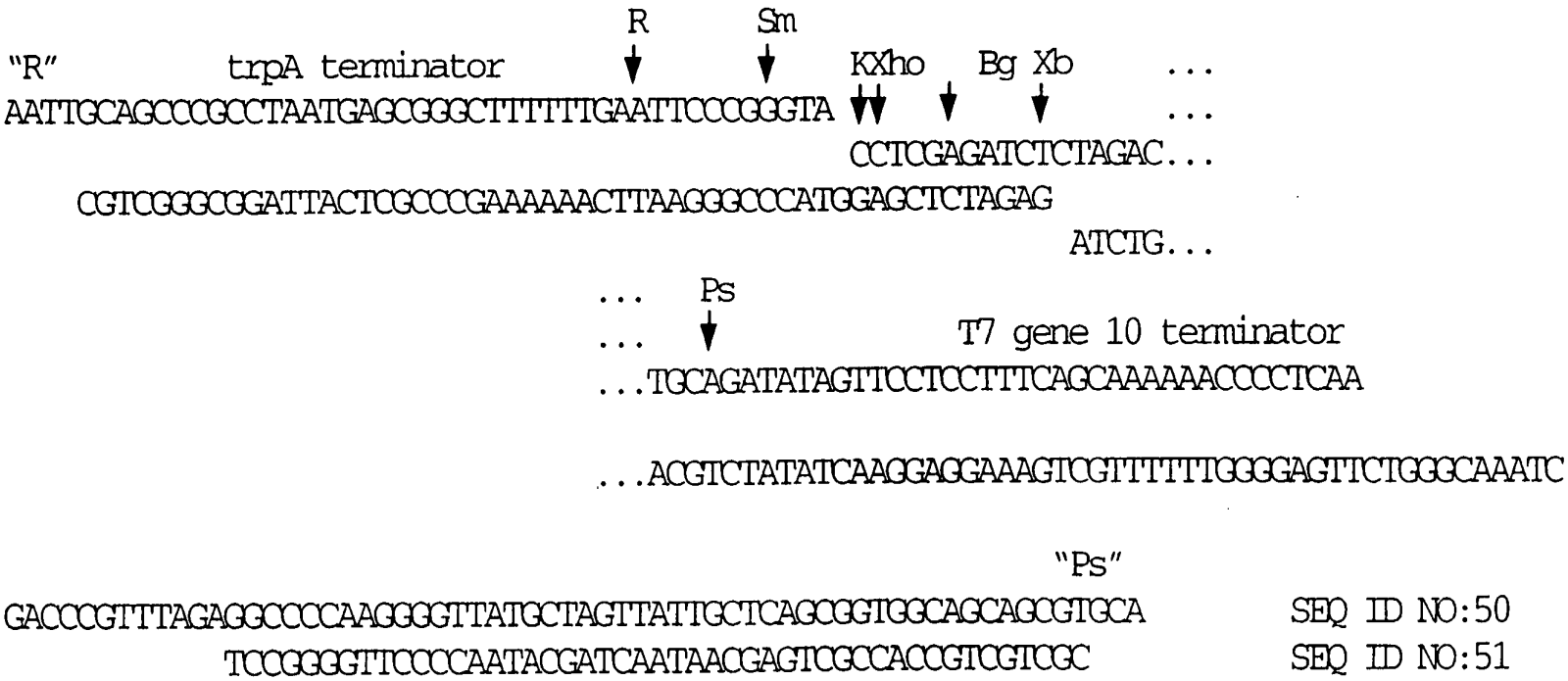


FIG.6A

FIG.6B

Oligonucleotides used to generate the multiple cloning site and transcription terminators for the expression plasmids



Construction of DS-2242-1 and DS-242-2, plasmids containing T7 hia (33) and cer.

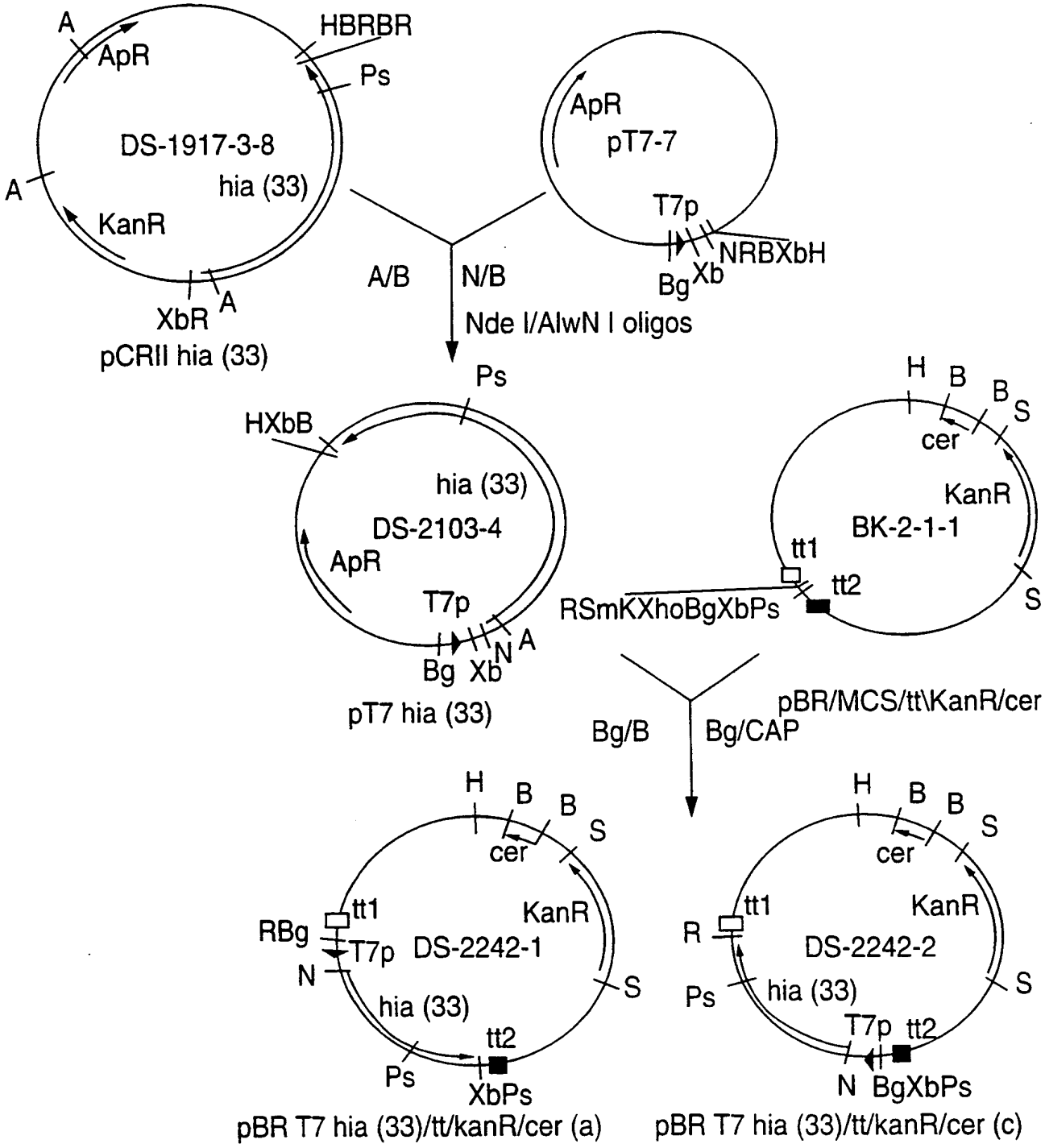


FIG.7A

FIG.7B

Oligonucleotides used to generate the 5'-end of the strain 33 *hia* gene for expression studies.

Nde I

M N K I F N V I W N V M T Q T W A V V S E L T R A H T K...

TATGAACAAAATTTTTAACGTTATTTGAATGTTATGACTCAAACTTGGGCTGTCG

TATCTGAACTCACTCGCGCCCACACCA...

ACTTGTTTTAAAAATTGCAATAAACCTTACAATACTGAGTTT

GAACCCGACAGCATAGACTTGAGTGAGCGCGGGTGTGGT...

...

... R A S A T V A A SEQ ID NO:54

...AACGTGCCTCCGCAACCGTGGCAGCCG SEQ ID NO:52

...TTGCACGGAGGCGTTGGCACCGTC SEQ ID NO:53

AlwN I

...

Construction of DS-2340-2-3,
a plasmid containing T7 V38 hia (33) and cer.

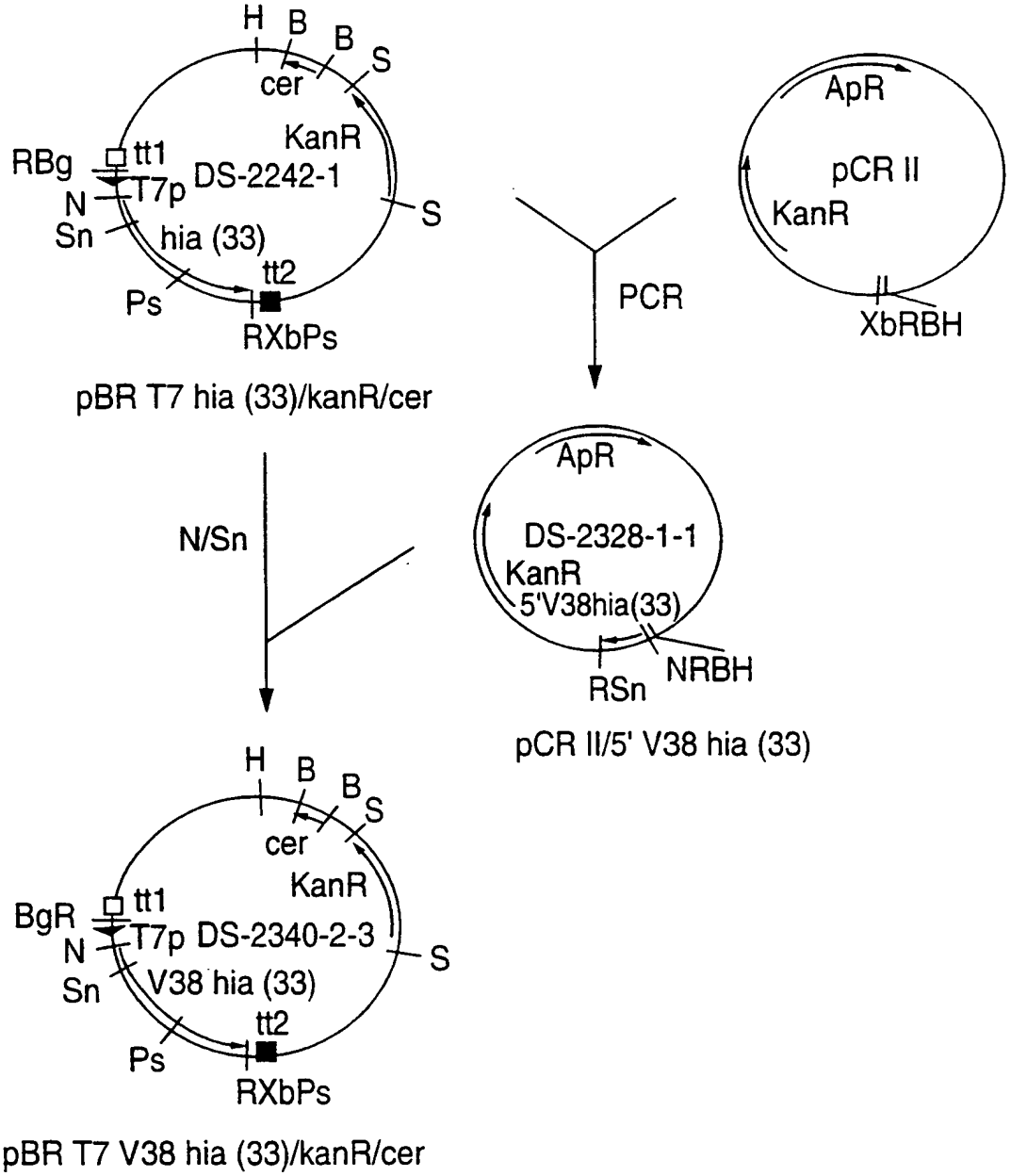


FIG.8A

FIG.8B

Oligonucleotides used to PCR amplify the strain 33 hia gene from the V38 codon to the SnaB I site.

sense

Nde I

M V L A T V L S A T

5′ GGGAATTCATATGGTATTGGCGACCGTATTGTCTGCAACG 3′ 6286.SL SEQ ID NO:60

SEQ ID NO:61

antisense

SnaB I

D E T T A T V G N L R K L

5′ GACGAAACCACCGCAACCGTAGGCAATTTACGTAAATTGAAGCTTCG 3′ SEQ ID NO:19

3′ CTGCTTTGGTGGCGTTGGCATCCGTTAAATGCATTTAACTTCGAAGC 5′ 6287.SL SEQ ID NO:18

SEQ ID NO:20

Construction of DS-2447-2,
a plasmid containing tandem T7 V38 hia (11) cassettes and cer.

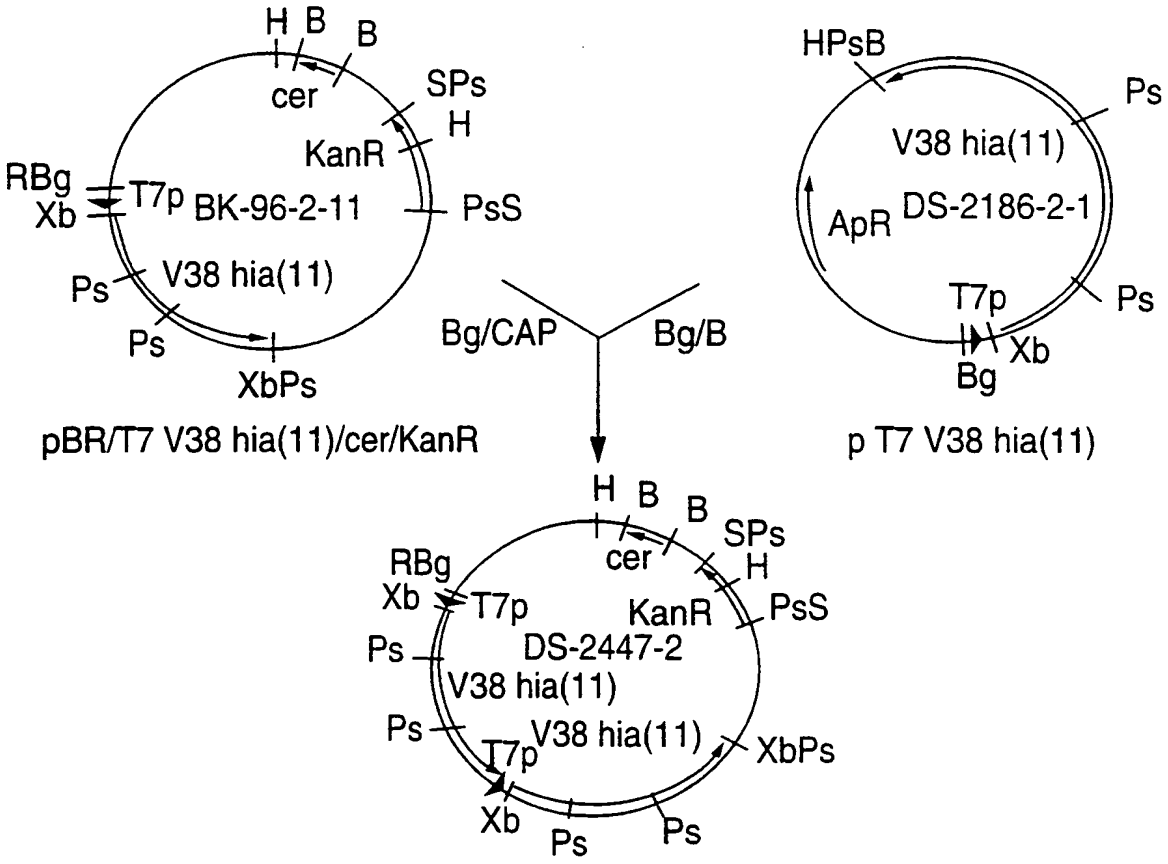


FIG.9A

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Construction of DS-2448-17,
a plasmid containing tandem T7 V38 hia(33) cassettes and cer.

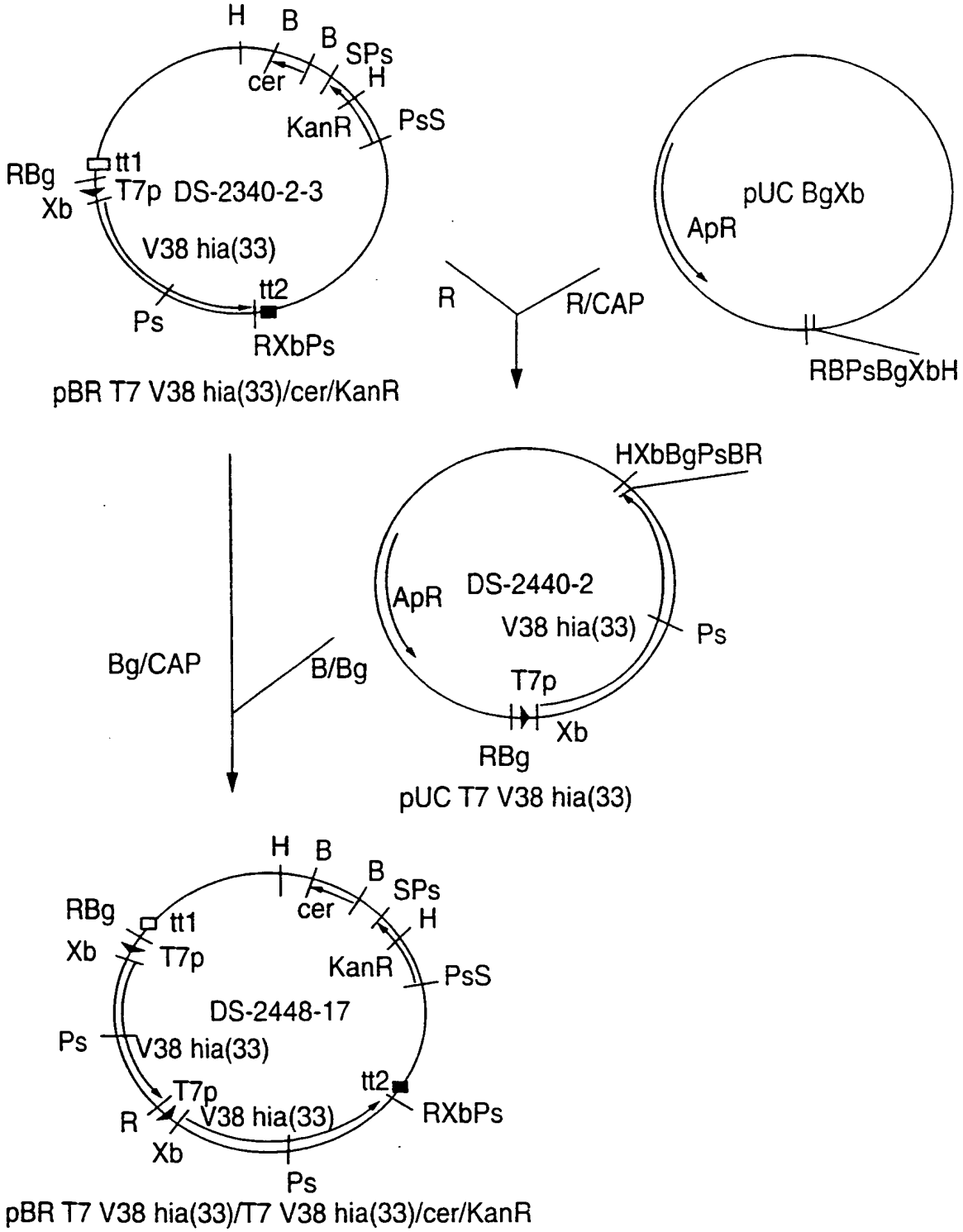
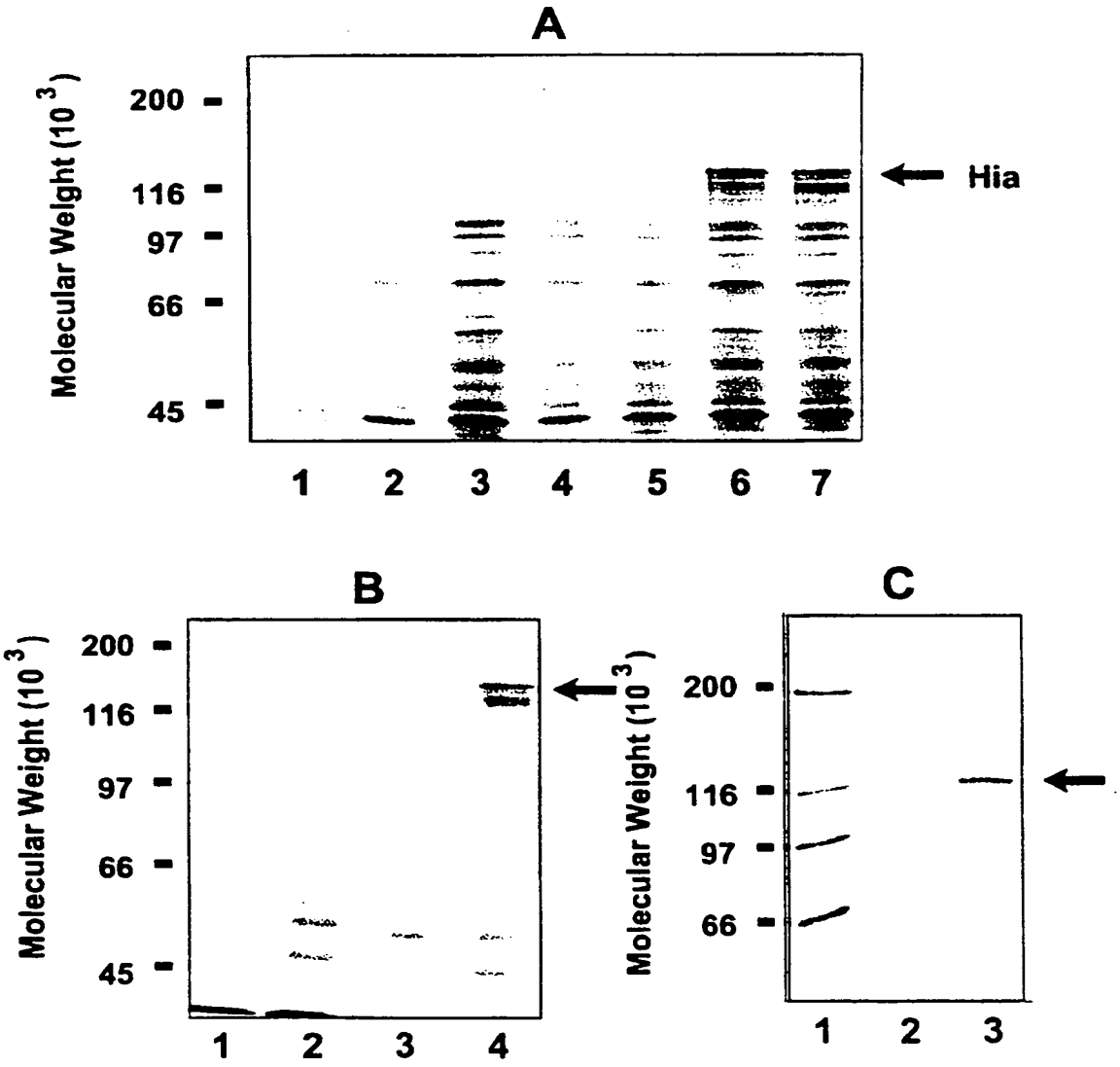


FIG.9B

FIG.10



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Purification of rHia Proteins from E. coli

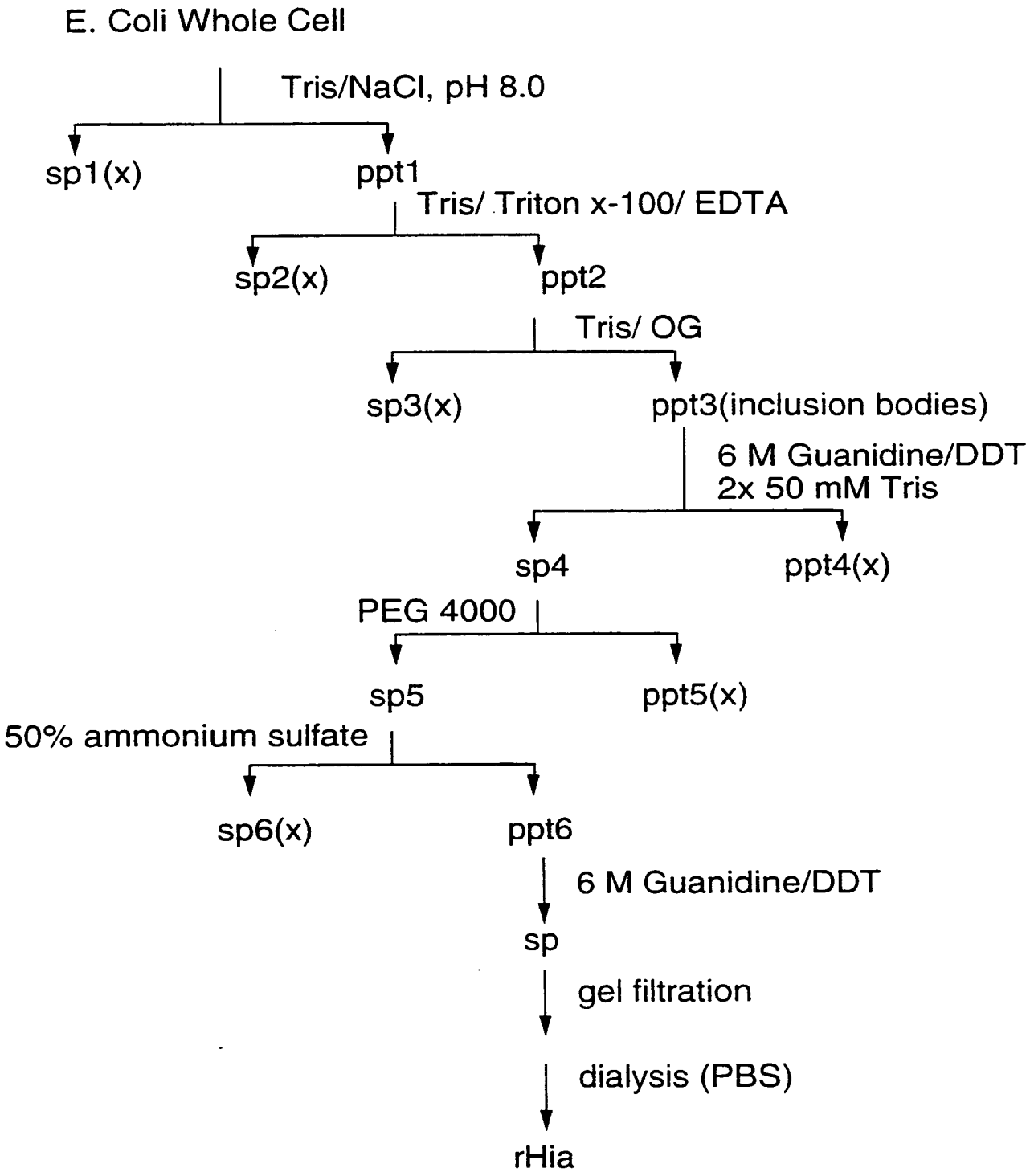
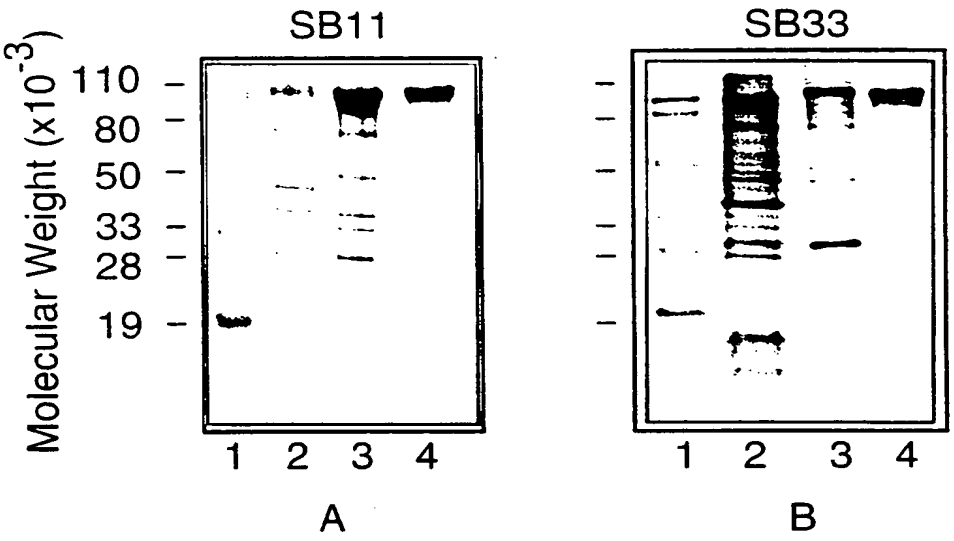


FIG.11

Purification of rHia (V38) from E. coli



1. Prestained molecular weight markers
2. E. coli whole cell lysate
3. Crude extract
4. Purified rHia protein

FIG.12

The Stability of rHia (V38/SB11)

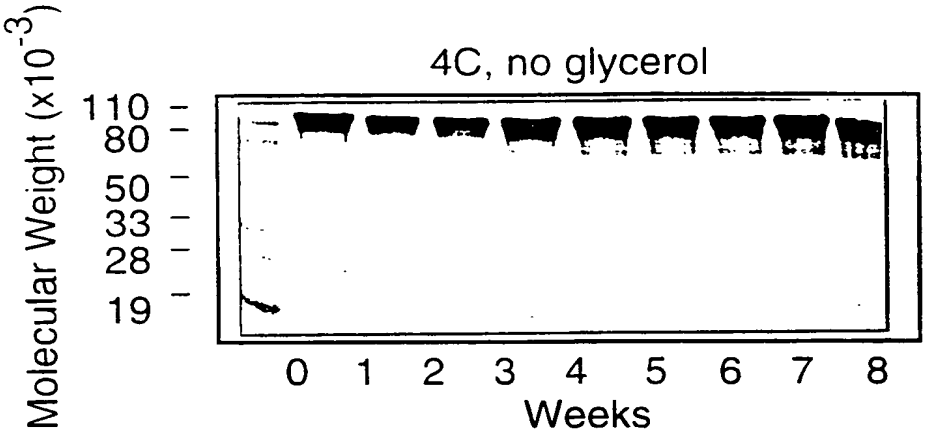


FIG.13A

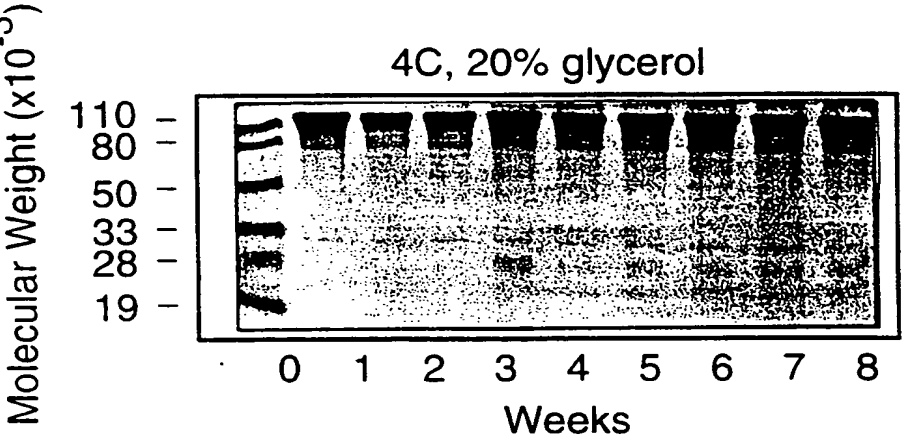


FIG.13B

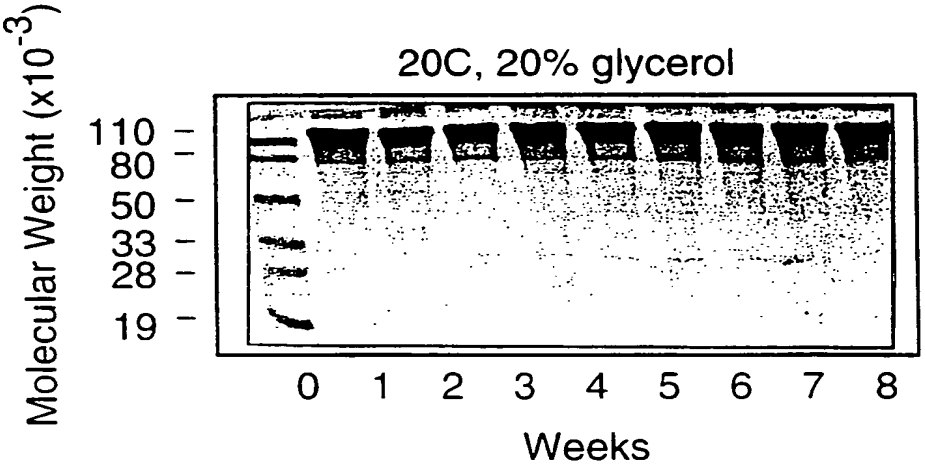


FIG.13C

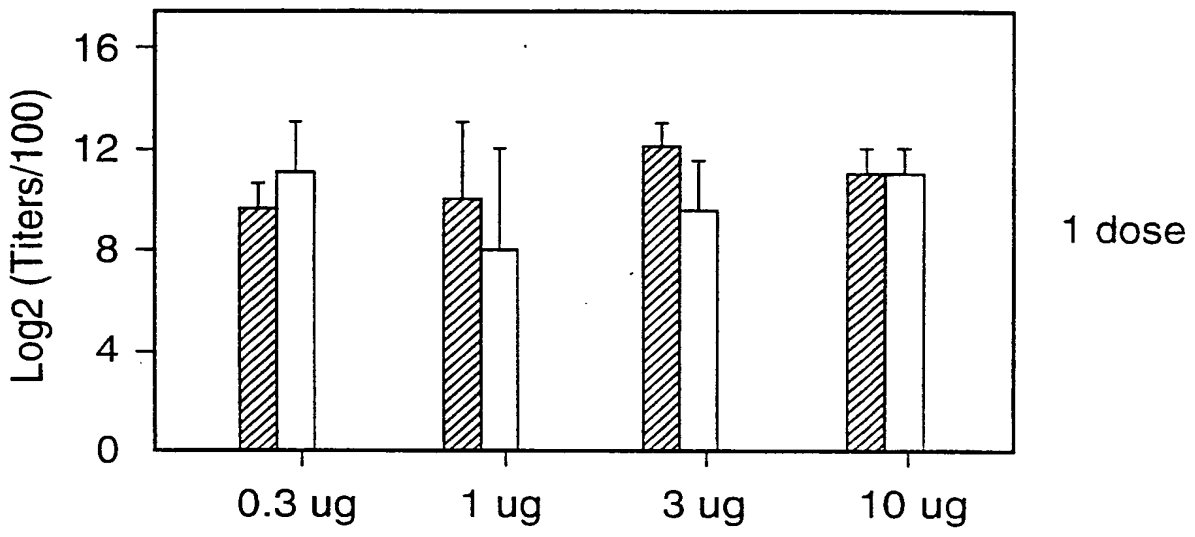


FIG.14A

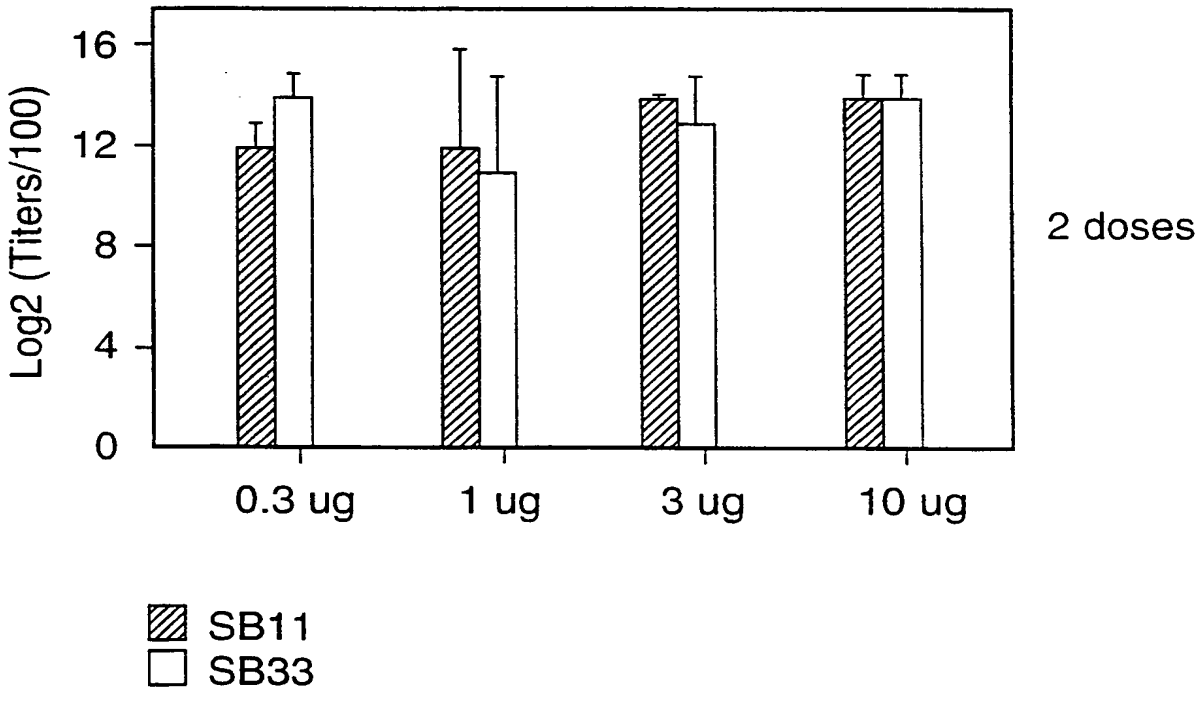


FIG.14B

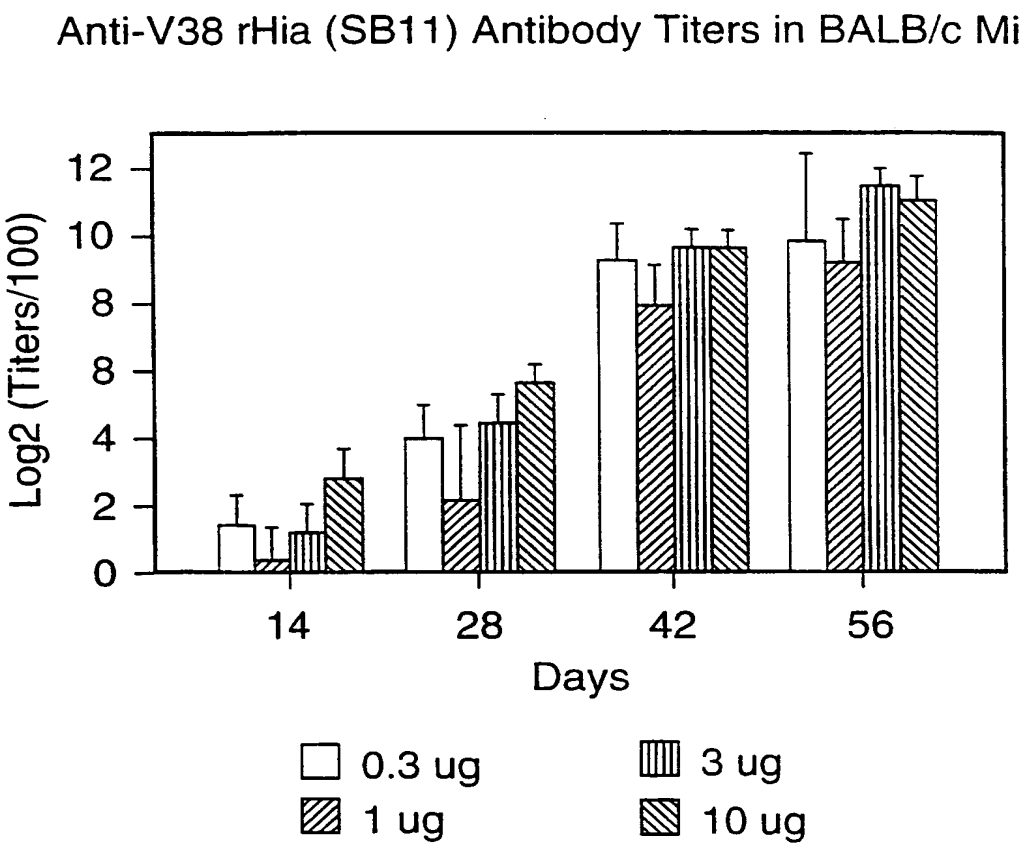


FIG.15A

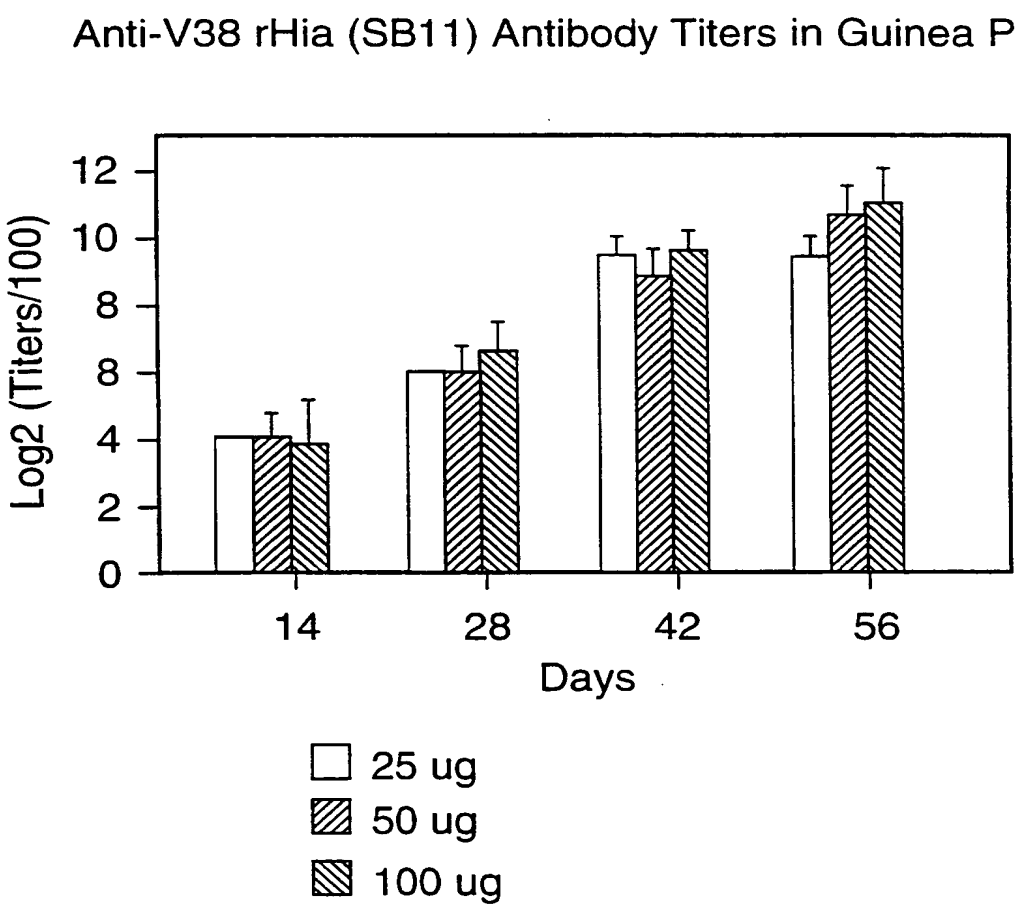


FIG.15B

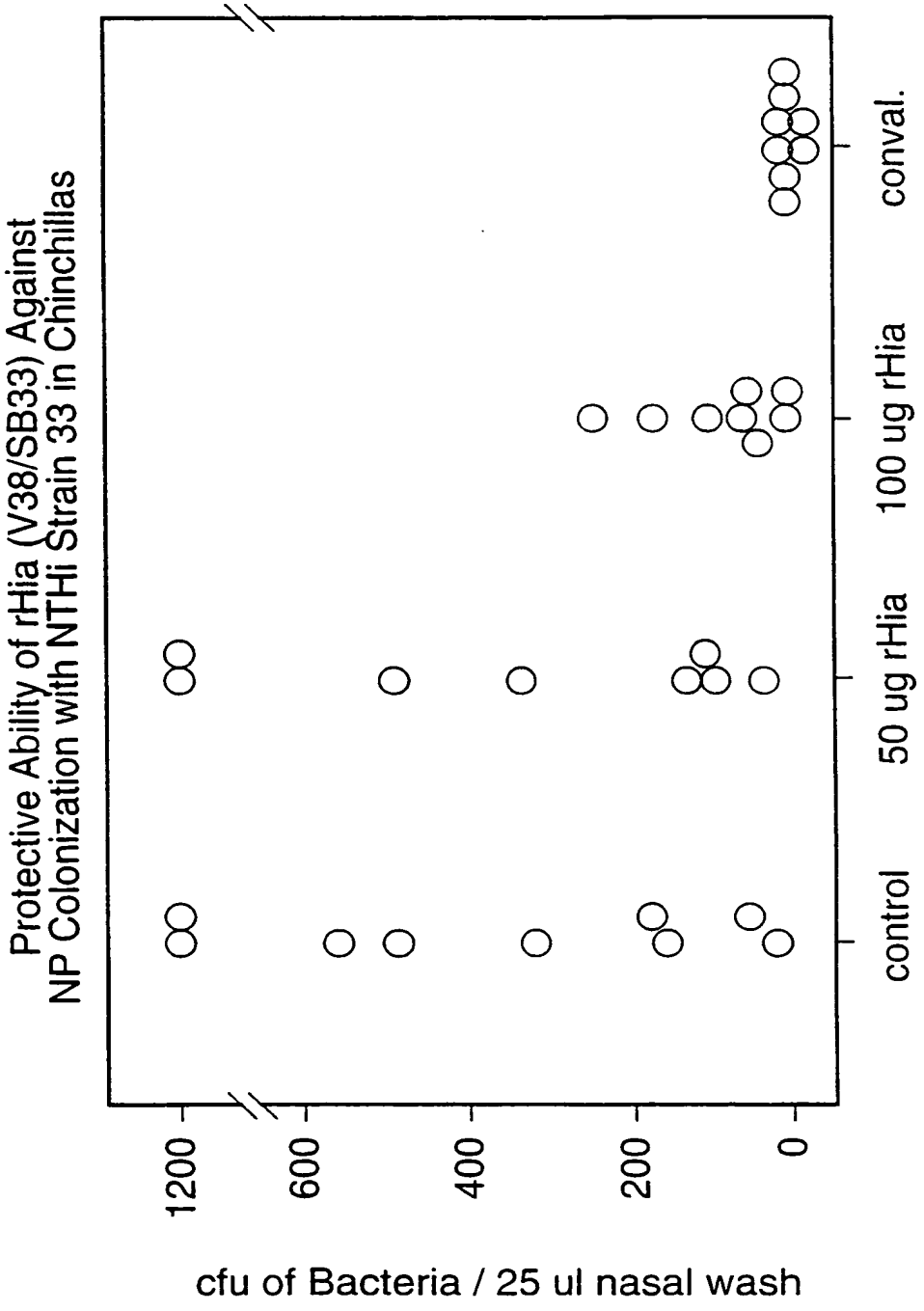


FIG.16

FIG.17

Oligonucleotides used to PCR amplify additional *hia* genes.

sense

M N K I F N V SEQ ID NO:22

5′ TTAAATATAAGGTAAATAAAAATGAACAAAATTTTTAACGTT 3′ 5040.SL SEQ ID NO:21

antisense

K T G V A A G V G Y Q W * * SEQ ID NO:5

5′ AAAACAGGCGTTGCAGCAGGTGTTGGTTACCAGTGGTAATAG 3′ SEQ ID NO:4

3′ TTTTGTCCGCAACGTCGTCCACAACCAATGGTCACCATTATCTTAAGGCCTAGGCG 5′ 5039.SL SEQ ID NO:3

EcoR I BamH I

FIG.18A

NTHi strain 33 Hia

```
                                        MET  ASN  LYS...
G A A T T C G G C T T A A A T A A A A A T G A A C A A ...
              10                  20              ...
                                ...  ILE  PHE  ASN  VAL  ILE  TRP  ASN  VAL  MET  THR  GLN
                                ...A A T T T T T A A C G T T A T T T G G A A T G T T A T G A C T C A
                                ...  30                  40                  50                  60

  THR  TRP  ALA  VAL  VAL  SER  GLU  LEU  THR...
A A C T T G G G C T G T C G T A T C T G A A C T C A C ...
              70                  80              ...
                                ...  ARG  ALA  HIS  THR  LYS  ARG  ALA  SER  ALA  THR  VAL
                                ...T C G C G C C C A C A C C A A A C G T G C C T C C G C A A C C G T
                                ...  90                  100                 110                 120

  ALA  ALA  ALA  VAL  LEU  ALA  THR  VAL  LEU...
G G C A G C C G C T G T A T T G G C G A C C G T A T T ...
              130                 140             ...
                                ...  SER  ALA  THR  VAL  GLN  ALA  SER  ALA  GLY  SER  THR
                                ...G T C T G C A A C G G T T C A G G C G A G T G C A G G C A G T A C
                                ...  150                 160                 170                 180

  THR  GLY  THR  ASN  SER  LEU  ASN  VAL  TYR...
G A C A G G T A C A A A T A G T T T G A A T G T T T A ...
              190                 200             ...
```

FIG.18B

```
                                    ...  GLY  LYS  ASN  ASN  SER  ASN  PHE  ASN  SER  ALA  ASN
                                    ...T G G A A A G A A T A A T T C G A A T T T C A A T T C A G C C A A
                                    ...  210                  220                  230                  240

  ASN  SER  ILE  ALA  ASP  LEU  ASN  LYS  GLN...
T A A T T C A A T A G C A G A T T T A A A T A A A C A ...
             250                  260                  ...
                                    ...  ASN  ASP  SER  VAL  TYR  ASP  GLY  LEU  LEU  ASN  LEU
                                    ...A A A T G A T A G T G T T T A C G A T G G T T T A T T A A A T C T
                                    ...  270                  280                  290                  300

  ASN  GLU  LYS  GLY  THR  ASP  LYS  SER  LYS...
G A A T G A A A A A G G T A C G G A T A A G T C A A A ...
             310                  320                  ...
                                    ...  PHE  LEU  VAL  ALA  ASP  GLU  THR  THR  ALA  THR  VAL
                                    ...A T T C C T G G T T G C T G A C G A A A C C A C C G C A A C C G T
                                    ...  330                  340                  350                  360

  GLY  ASN  LEU  ARG  LYS  LEU  GLY  TRP  VAL...
A G G C A A T T T A C G T A A A T T G G G T T G G G T ...
             370                  380                  ...
                                    ...  VAL  SER  THR  LYS  ASN  SER  THR  LYS  GLU  GLU  SER
                                    ...A G T A T C A A C C A A A A A C A G T A C G A A A G A A G A A A G
                                    ...  390                  400                  410                  420
```

FIG.18C

```
   ASN  GLN  VAL  LYS  GLN  ALA  ASP  GLU  VAL...
C A A T C A A G T C A A A C A G G C G G A T G A A G T ...
            430              440              ...
                                          ...  LEU  PHE  GLU  GLY  LYS  ASP  GLY  VAL  THR  VAL  THR
                                          ...G T T G T T T G A A G G C A A A G A C G G T G T A A C G G T T A C
                                          ...  450              460              470              480

   SER  LYS  SER  GLU  ASN  GLY  LYS  HIS  THR...
T T C C A A A T C T G A A A A C G G C A A A C A C A C ...
            490              500              ...
                                          ...  VAL  THR  PHE  ALA  LEU  ALA  ASN  ASP  LEU  ASN  VAL
                                          ...C G T T A C T T T T G C C C T T G C G A A T G A C C T T A A T G T
                                          ...  510              520              530              540

   LYS  ASN  ALA  THR  VAL  SER  ASP  LYS  LEU...
A A A A A A C G C A A C C G T T A G C G A T A A A T T ...
            550              560              ...
                                          ...  SER  LEU  GLY  ALA  ASN  GLY  LYS  LYS  VAL  ASP  ILE
                                          ...A T C G C T T G G T G C A A A C G G C A A G A A A G T C G A T A T
                                          ...  570              580              590              600

   THR  SER  ASP  ALA  ASN  GLY  LEU  LYS  PHE...
T A C C A G T G A T G C A A A C G G C T T G A A A T T ...
            610              620              ...
```

FIG.18D

```
                                    ...  ALA  LYS  GLN  GLY  THR  ASN  GLY  GLN  ASN  GLY  ASN
                                    ...T G C G A A A C A G G G T A C G A A T G G T C A A A A C G G T A A
                                    ...  630                  640                  650                  660

  VAL  HIS  LEU  ASN  GLY  ILE  ALA  SER  THR...
T G T T C A C T T A A A C G G T A T T G C T T C G A C ...
              670                  680                  ...
                                    ...  LEU  ASP  ASP  PRO  ARG  VAL  GLY  GLY  LYS  THR  ALA
                                    ...T T T A G A T G A T C C T C G T G T G G G T G G A A A A A C A G C
                                    ...  690                  700                  710                  720

  HIS  LEU  THR  LYS  GLU  ILE  SER  ASP  THR...
A C A C C T T A C A A A A G A A A T C A G C G A T A C ...
              730                  740                  ...
                                    ...  GLU  ARG  ASN  ARG  ALA  ALA  SER  VAL  GLY  ASP  VAL
                                    ...A G A A C G T A A C C G T G C T G C G A G C G T G G G C G A T G T
                                    ...  750                  760                  770                  780

  LEU  ASN  ALA  GLY  TRP  ASN  ILE  ARG  GLY...
A T T G A A T G C G G G T T G G A A T A T T C G T G G ...
              790                  800                  ...
                                    ...  ALA  LYS  THR  ILE  GLY  GLY  THR  VAL  ASP  ASN  VAL
                                    ...C G C A A A A A C G A T T G G C G G T A C A G T G G A T A A T G T
                                    ...  810                  820                  830                  840
```

FIG.18E

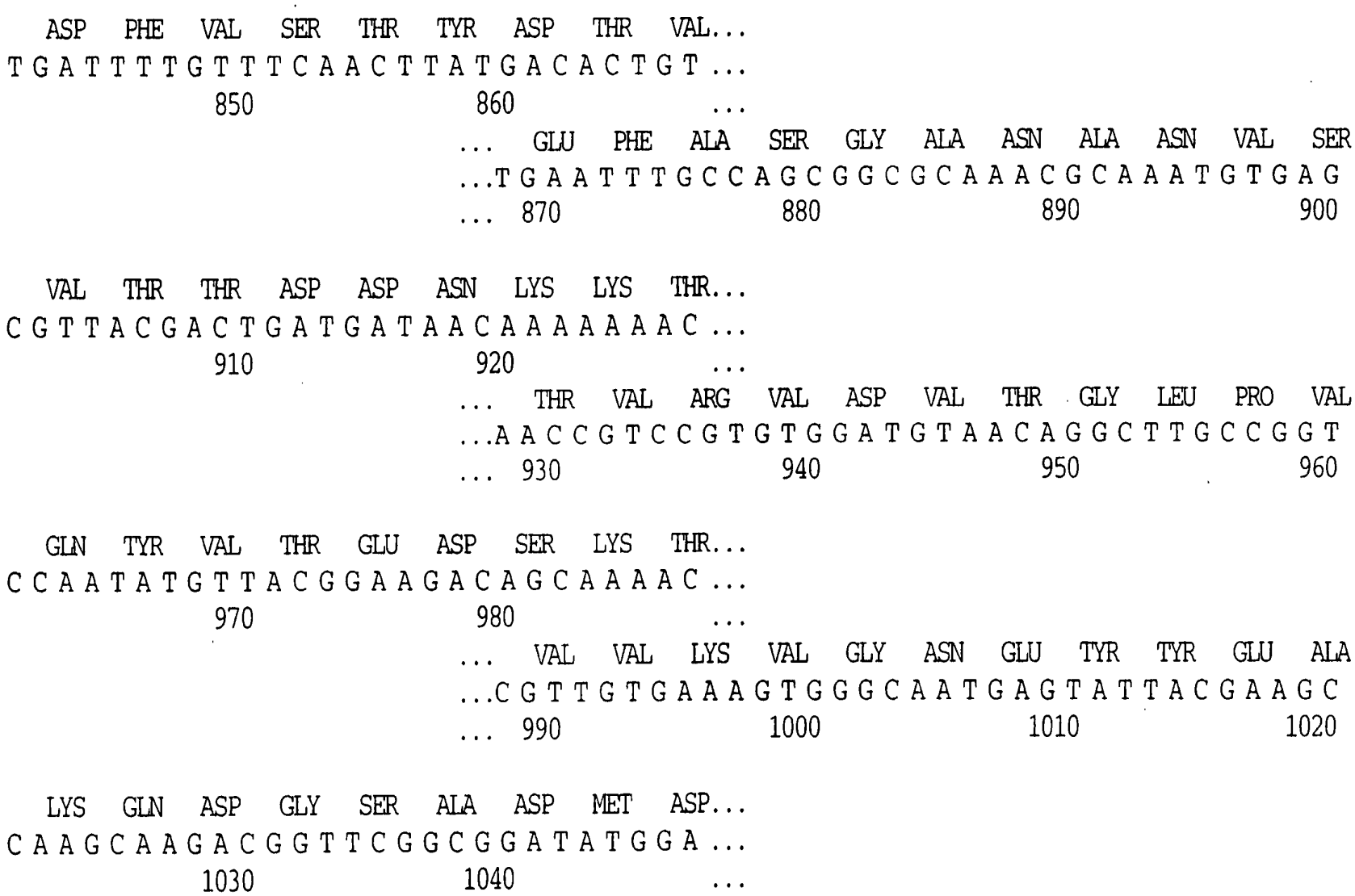


FIG.18F

```
                                ... LYS LYS VAL GLU ASN GLY LYS LEU ALA LYS THR
                                ...T A A A A A A G T C G A A A A T G G C A A G C T G G C G A A A A C
                                ... 1050          1060          1070          1080

  LYS VAL LYS LEU VAL SER ALA ASN GLY...
T A A A G T G A A A T T G G T A T C G G C A A A C G G ...
          1090          1100          ...
                                ... THR ASN PRO VAL LYS ILE SER ASN VAL ALA ASP
                                ...T A C A A A T C C G G T G A A A A T C A G C A A T G T T G C G G A
                                ... 1110          1120          1130          1140

  GLY THR GLU ASP THR ASP ALA VAL SER...
C G G C A C G G A A G A T A C C G A T G C G G T C A G ...
          1150          1160          ...
                                ... PHE LYS GLN LEU LYS ALA LEU GLN ASP LYS GLN
                                ...C T T T A A G C A G T T G A A A G C C T T G C A A G A T A A A C A
                                ... 1170          1180          1190          1200

  VAL THR LEU SER ALA SER ASN ALA TYR...
G G T T A C G T T A A G T G C G A G C A A T G C T T A ...
          1210          1220          ...
                                ... ALA ASN GLY GLY SER ASP ALA ASP GLY GLY LYS
                                ...T G C C A A T G G C G G T A G C G A T G C C G A C G G C G G C A A
                                ... 1230          1240          1250          1260
```

FIG.18G

```
   ALA  THR  GLN  THR  LEU  GLY  ASN  ASP  LEU...
GGCAACTCAAACTTTAGGCAATGATTT...
          1270           1280           ...
                                ...  ASN  PHE  LYS  PHE  LYS  SER  THR  ASP  SER  GLU  LEU
                                ...GAATTTTAAATTTAAATCCACAGACAGCGAGTT
                                ... 1290           1300           1310           1320

   LEU  ASN  ILE  LYS  ALA  ALA  GLY  ASP  THR...
GTTGAACATCAAAGCAGCAGGTGACAC...
          1330           1340           ...
                                ...  VAL  THR  PHE  THR  PRO  LYS  LYS  GLY  SER  VAL  GLN
                                ...GGTTACCTTTACGCCGAAAAAAGGTTCGGTGCA
                                ... 1350           1360           1370           1380

   VAL  GLY  ASP  ASP  GLY  LYS  ALA  THR  ILE...
GGTTGGCGATGATGGTAAGGCTACGAT...
          1390           1400           ...
                                ...  GLN  ASP  GLY  ALA  LYS  THR  THR  THR  GLY  LEU  VAL
                                ...TCAAGACGGCGCGAAAACAACTACCGGTTTGGT
                                ... 1410           1420           1430           1440

   GLU  ALA  SER  GLU  LEU  VAL  ASP  SER  LEU...
TGAGGCTTCTGAATTGGTTGACAGCCT...
          1450           1460           ...
```

FIG.18H

```
                                    ...  ASN  LYS  LEU  GLY  TRP  LYS  VAL  GLY  VAL  GLY  LYS
                                    ...G A A C A A A T T G G G C T G G A A A G T G G G C G T T G G T A A
                                    ... 1470              1480              1490              1500

  ASP  GLY  THR  GLY  ALA  THR  ASP  GLY  THR...
A G A C G G C A C A G G A G C G A C C G A T G G C A C ...
             1510              1520              ...
                                    ...  HIS  THR  ASP  THR  LEU  VAL  LYS  SER  GLY  ASP  LYS
                                    ...G C A T A C C G A C A C T T T A G T G A A G T C G G G C G A T A A
                                    ... 1530              1540              1550              1560

  VAL  THR  LEU  LYS  ALA  GLY  ASP  ASN  LEU...
A G T A A C T T T G A A A G C C G G C G A T A A T C T ...
             1570              1580              ...
                                    ...  LYS  VAL  LYS  GLN  GLU  GLY  THR  ASN  PHE  THR  TYR
                                    ...G A A G G T C A A A C A A G A G G G T A C A A A C T T C A C T T A
                                    ... 1590              1600              1610              1620

  VAL  LEU  ARG  ASP  GLU  LEU  THR  GLY  VAL...
C G T G C T C A G A G A T G A A T T G A C G G G C G T ...
             1630              1640              ...
                                    ...  LYS  SER  VAL  GLU  PHE  LYS  ASP  THR  GLU  ASN  GLY
                                    ...A A A G A G C G T G G A G T T T A A A G A C A C G G A G A A T G G
                                    ... 1650              1660              1670              1680
```

FIG.18I

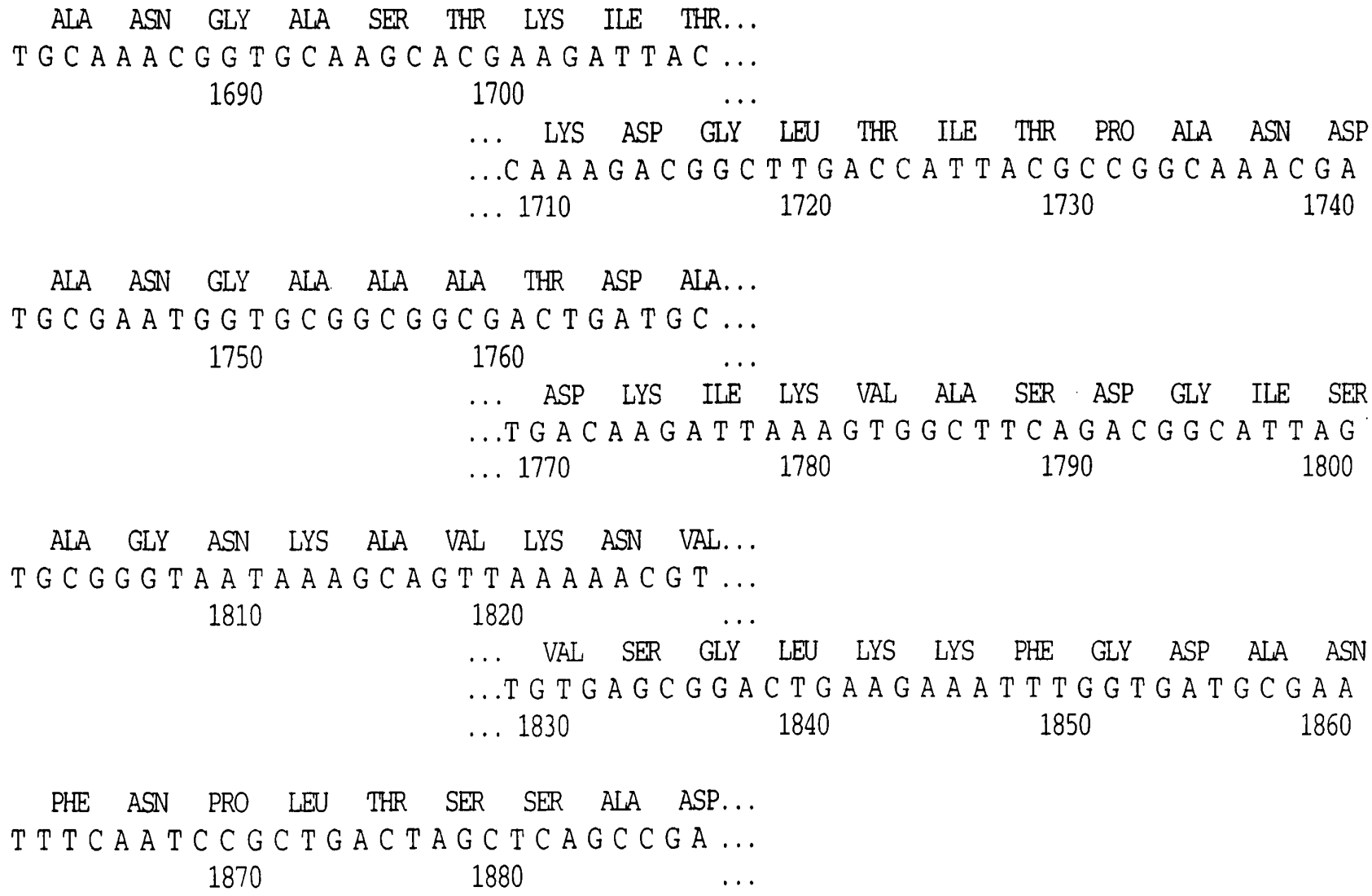


FIG.18J

```
                                    ...  ASN  LEU  THR  LYS  GLN  TYR  ASP  ASN  ALA  TYR  LYS
                                    ...C A A C T T A A C G A A A C A A T A T G A C A A T G C C T A T A A
                                    ... 1890              1900              1910              1920

  GLY  LEU  THR  ASN  LEU  ASP  GLU  LYS  SER...
A G G C T T G A C C A A T C T G G A T G A A A A A A G ...
            1930              1940              ...
                                    ...  LYS  GLY  LYS  GLN  THR  PRO  THR  VAL  ALA  ASP  ASN
                                    ...T A A A G G C A A G C A A A C T C C G A C C G T T G C T G A C A A
                                    ... 1950              1960              1970              1980

  THR  ALA  ALA  THR  VAL  GLY  ASP  LEU  ARG...
T A C C G C T G C A A C C G T G G G C G A T T T G C G ...
            1990              2000              ...
                                    ...  GLY  LEU  GLY  TRP  VAL  ILE  SER  ALA  ASP  LYS  THR
                                    ...C G G T T T G G G C T G G G T C A T T T C T G C A G A C A A A A C
                                    ... 2010              2020              2030              2040

  THR  GLY  GLU  SER  LYS  GLU  TYR  SER  ALA...
C A C A G G C G A G T C A A A G G A A T A T A G C G C ...
            2050              2060              ...
                                    ...  GLN  VAL  ARG  ASN  ALA  ASN  GLU  VAL  LYS  PHE  LYS
                                    ...G C A A G T G C G T A A C G C C A A T G A A G T G A A A T T C A A
                                    ... 2070              2080              2090              2100
```

FIG.18K

```
   SER  GLY  ASN  GLY  ILE  ASN  VAL  SER  GLY...
GAGCGGCAACGGTATCAATGTTTCCGG...
          2110          2120          ...
                        ...  LYS  THR  LEU  ASP  ASN  GLY  THR  ARG  GLU  ILE  THR
                        ...TAAAACATTGGATAACGGTACGCGCGAAATTAC
                        ... 2130          2140          2150          2160

   PHE  GLU  LEU  ALA  LYS  ASP  GLU  ASN  ALA...
TTTTGAATTGGCTAAAGACGAAAATGC...
          2170          2180          ...
                        ...  ILE  ALA  PHE  GLY  SER  GLY  SER  LYS  ALA  LEU  ARG
                        ...CATTGCTTTCGGTTCTGGCTCAAAAGCCTTGCG
                        ... 2190          2200          2210          2220

   ASP  ASN  THR  VAL  ALA  ILE  GLY  THR  GLY...
CGATAACACGGTGGCGATTGGTACGGG...
          2230          2240          ...
                        ...  ASN  VAL  VAL  ASN  ALA  GLU  LYS  SER  GLY  ALA  PHE
                        ...CAACGTTGTGAATGCGGAAAAATCTGGTGCATT
                        ... 2250          2260          2270          2280

   GLY  ASP  PRO  ASN  TYR  ILE  GLU  ASP  LYS...
CGGCGATCCGAACTACATCGAAGATAA...
          2290          2300          ...
```

FIG.18L

```
                                    ...  ALA  GLY  GLY  SER  TYR  ALA  PHE  GLY  ASN  ASP  ASN
                                    ...A G C C G G T G G C A G C T A C G C T T T C G G T A A C G A T A A
                                    ... 2310              2320              2330              2340

  ARG  ILE  THR  SER  LYS  ASN  THR  PHE  VAL...
C C G T A T T A C T T C T A A A A A C A C T T T T G T ...
            2350              2360              ...
                                    ...  LEU  GLY  ASN  GLY  VAL  ASN  ALA  LYS  TYR  LYS  ALA
                                    ...G T T G G G T A A T G G A G T T A A T G C G A A A T A T A A A G C
                                    ... 2370              2380              2390              2400

  ASN  GLY  ASP  VAL  ASP  THR  GLU  THR  VAL...
C A A T G G A G A T G T T G A T A C G G A A A C C G T ...
            2410              2420              ...
                                    ...  THR  VAL  LYS  ASP  LYS  ASP  GLY  LYS  GLU  THR  THR
                                    ...A A C T G T T A A G G A C A A A G A C G G T A A A G A G A C T A C
                                    ... 2430              2440              2450              2460

  VAL  THR  VAL  PRO  LYS  ALA  LEU  GLY  ALA...
C G T T A C T G T T C C T A A A G C G T T A G G G G C ...
            2470              2480              ...
                                    ...  THR  VAL  GLU  ASN  SER  VAL  TYR  LEU  GLY  ASN  LYS
                                    ...T A C G G T T G A A A A C T C C G T T T A T T T G G G T A A T A A
                                    ... 2490              2500              2510              2520
```

FIG.18M

```
   SER  THR  ALA  THR  LYS  ASP  LYS  GLY  LYS...
A T C G A C T G C G A C A A A A G A T A A G G G T A A ...
           2530           2540           ...
                                          ...  ASN  LEU  LYS  SER  ASP  GLY  THR  ALA  GLY  ASN  THR
                                          ...A A A T C T G A A A T C T G A T G G T A C G G C G G G T A A C A C
                                          ... 2550           2560           2570           2580

   THR  THR  ALA  GLY  THR  THR  GLY  THR  VAL...
T A C A A C T G C T G G T A C A A C G G G T A C G G T ...
           2590           2600           ...
                                          ...  ASN  GLY  PHE  ALA  GLY  ALA  THR  ALA  HIS  GLY  ALA
                                          ...A A A C G G C T T T G C C G G T G C A A C G G C G C A C G G T G C
                                          ... 2610           2620           2630           2640

   VAL  SER  VAL  GLY  ALA  SER  GLY  GLU  GLU...
G G T T T C T G T C G G C G C A A G C G G C G A A G A ...
           2650           2660           ...
                                          ...  ARG  ARG  ILE  GLN  ASN  VAL  ALA  ALA  GLY  GLU  ILE
                                          ...A A G A C G T A T C C A A A A C G T T G C G G C A G G C G A A A T
                                          ... 2670           2680           2690           2700

   SER  ALA  THR  SER  THR  ASP  ALA  ILE  ASN...
T T C C G C T A C T T C C A C C G A T G C G A T T A A ...
           2710           2720           ...
```

FIG.18N

```
                                    ...  GLY  SER  GLN  LEU  TYR  ALA  VAL  ALA  LYS  GLY  VAL
                                    ...C G G C A G C C A G T T G T A T G C C G T G G C A A A A G G G G T
                                    ... 2730              2740              2750              2760

  THR  ASN  LEU  ALA  GLY  GLN  VAL  ASN  LYS...
A A C A A A C C T T G C T G G A C A A G T G A A T A A ...
            2770              2780              ...
                                    ...  VAL  GLY  LYS  ARG  ALA  ASP  ALA  GLY  THR  ALA  SER
                                    ...A G T G G G C A A A C G T G C A G A T G C A G G T A C A G C A A G
                                    ... 2790              2800              2810              2820

  ALA  LEU  ALA  ALA  SER  GLN  LEU  PRO  GLN...
T G C A T T A G C G G C T T C A C A G T T A C C A C A ...
            2830              2840              ...
                                    ...  ALA  SER  MET  SER  GLY  LYS  SER  MET  VAL  SER  ILE
                                    ...A G C C T C T A T G T C A G G T A A A T C A A T G G T T T C T A T
                                    ... 2850              2860              2870              2880

  ALA  GLY  SER  SER  TYR  GLN  GLY  GLN  SER...
T G C G G G A A G T A G T T A T C A A G G T C A A A G ...
            2890              2900              ...
                                    ...  GLY  LEU  ALA  ILE  GLY  VAL  SER  ARG  ILE  SER  ASP
                                    ...T G G T T T A G C T A T C G G G G T A T C A A G A A T T T C C G A
                                    ... 2910              2920              2930              2940
```

FIG.18O

```
  ASN   GLY   LYS   VAL   ILE   ILE   ARG   LEU   SER...
T A A T G G C A A A G T G A T T A T T C G C T T G T C ...
              2950                2960                ...
                             ...  GLY   THR   THR   ASN   SER   GLN   GLY   LYS   THR   GLY   VAL
                             ...A G G C A C A A C C A A T A G C C A A G G T A A A A C A G G C G T
                             ... 2970                2980                2990                3000

  ALA   ALA   GLY   VAL   GLY   TYR   GLN   TRP   ***
T G C A G C A G G T G T T G G T T A C C A G T G G T A ...
              3010                3020                ...

                             ...A T A G A A T T C
                             ... 3030
```

FIG.19A

NTHi strain 32 *hia*

```
G A A T T C G G C T T T A A A T A T A A G G T A A A T A A A ...
              10                  20                  30 ...
                                    MET  ASN  LYS  ILE  PHE  ASN  VAL  ILE  TRP  ASN
                                ...A A T G A A C A A A A T T T T T A A C G T T A T T T G G A A
                                ...                 40                  50                  60

   VAL  VAL  THR  GLN  THR  TRP  VAL  VAL  VAL  SER...
T G T T G T G A C T C A A A C T T G G G T T G T C G T A T C ...
              70                  80                  90 ...
                                ...  GLU  LEU  THR  ARG  THR  HIS  THR  LYS  CYS  ALA
                                ...T G A A C T C A C T C G C A C C C A C A C C A A A T G C G C
                                ...                 100                 110                 120

   SER  ALA  THR  VAL  ALA  VAL  ALA  VAL  LEU  ALA...
C T C C G C C A C C G T G G C A G T T G C C G T A T T G G C ...
              130                 140                 150 ...
                                ...  THR  LEU  LEU  SER  ALA  THR  VAL  GLN  ALA  ASN
                                ...A A C C C T G T T G T C C G C A A C G G T T C A G G C G A A
                                ...                 160                 170                 180

   ALA  THR  ASP  GLU  ASN  GLU  ASP  ASP  GLU  GLU...
T G C T A C C G A T G A A A A C G A A G A T G A T G A A G A ...
              190                 200                 210 ...
```

FIG.19B

```
                                    ...  GLU  LEU  GLU  PRO  VAL  GLN  ARG  SER  VAL  LEU
                                    ...A G A G T T A G A A C C C G T A C A A C G C T C T G T T T T
                                    ...              220              230              240

  ARG  TRP  SER  PHE  LYS  SER  ALA  LYS  GLU  GLY...
A A G G T G G A G C T T C A A A T C C G C T A A G G A A G G ...
            250              260              270 ...
                                    ...  THR  GLY  GLU  GLN  GLU  GLY  THR  THR  GLU  VAL
                                    ...C A C T G G A G A A C A A G A G G G A A C A A C A G A G G T
                                    ...              280              290              300

  ILE  ASN  LEU  ASN  THR  ASP  SER  SER  GLY  ASN...
A A T A A A T T T G A A C A C A G A T T C A T C A G G A A A ...
            310              320              330 ...
                                    ...  ALA  VAL  GLY  SER  SER  THR  ILE  THR  PHE  LYS
                                    ...T G C A G T A G G A A G C A G C A C A A T C A C C T T C A A
                                    ...              340              350              360

  ALA  GLY  ASP  ASN  LEU  LYS  ILE  LYS  GLN  SER...
A G C C G G C G A C A A C C T G A A A A T C A A A C A A A G ...
            370              380              390 ...
                                    ...  GLY  ASN  ASP  PHE  THR  TYR  SER  LEU  LYS  LYS
                                    ...C G G C A A T G A C T T C A C C T A C T C G C T G A A A A A
                                    ...              400              410              420
```

FIG.19C

```
   GLU   LEU   LYS   ASN   LEU   THR   SER   VAL   GLU   THR...
A G A G C T G A A A A A C C T G A C C A G T G T T G A A A C ...
               430                 440                 450 ...
                                  ...   GLU   LYS   LEU   SER   PHE   GLY   ALA   ASN   GLY   ASN
                                  ...T G A A A A A T T A T C G T T T G G C G C A A A C G G C A A
                                  ...                 460                 470                 480

   LYS   VAL   ASP   ILE   THR   SER   ASP   ALA   ASN   GLY...
T A A A G T T G A T A T T A C C A G T G A T G C A A A T G G ...
               490                 500                 510 ...
                                  ...   LEU   LYS   LEU   ALA   LYS   THR   GLY   ASN   GLY   ASN
                                  ...C T T G A A A T T G G C G A A A A C A G G T A A C G G A A A
                                  ...                 520                 530                 540

   GLY   GLN   ASN   SER   ASN   VAL   HIS   LEU   ASN   GLY...
T G G T C A A A A C A G T A A T G T T C A C T T A A A C G G ...
               550                 560                 570 ...
                                  ...   ILE   ALA   SER   THR   LEU   THR   ASP   THR   LEU   ALA
                                  ...T A T T G C T T C G A C T T T G A C C G A T A C G C T T G C
                                  ...                 580                 590                 600

   GLY   GLY   THR   THR   GLY   HIS   VAL   ASP   THR   ASN...
C G G T G G C A C A A C A G G A C A C G T T G A C A C C A A ...
               610                 620                 630 ...
```

FIG.19D

```
                                    ...  ILE  ASP  ALA  VAL  ASN  TYR  HIS  ARG  ALA  ALA
                                    ...C A T T G A T G C G G T T A A T T A T C A T C G C G C T G C
                                    ...              640                650                660

  SER  VAL  GLN  ASP  VAL  LEU  ASN  SER  GLY  TRP...
A A G C G T A C A A G A T G T G T T A A A C A G C G G T T G ...
              670                680                690 ...
                                    ...  ASN  ILE  GLN  GLY  ASN  GLY  ASN  ASN  VAL  ASP
                                    ...G A A T A T C C A A G G C A A T G G A A A C A A T G T C G A
                                    ...              700                710                720

  PHE  VAL  ARG  THR  TYR  ASP  THR  VAL  ASP  PHE...
T T T T G T C C G T A C T T A C G A C A C C G T G G A C T T ...
              730                740                750 ...
                                    ...  VAL  ASN  GLY  ALA  ASN  ALA  ASN  VAL  SER  VAL
                                    ...T G T C A A T G G C G C G A A T G C C A A T G T G A G C G T
                                    ...              760                770                780

  THR  ALA  ASP  THR  ALA  HIS  LYS  LYS  THR  THR...
T A C G G C T G A T A C G G C T C A C A A A A A G A C A A C ...
              790                800                810 ...
                                    ...  VAL  ARG  VAL  ASP  VAL  THR  GLY  LEU  PRO  VAL
                                    ...T G T C C G T G T G G A T G T A A C A G G C T T G C C G G T
                                    ...              820                830                840
```

FIG.19E

```
   GLN   TYR   VAL   THR   GLU   ASP   GLY   LYS   THR   VAL...
T C A A T A T G T T A C G G A A G A C G G C A A A A C C G T ...
               850                 860                 870 ...
                                  ...   VAL   LYS   VAL   GLY   ASN   GLU   TYR   TYR   LYS   ALA
                                  ...T G T G A A A G T G G G C A A T G A G T A T T A C A A A G C
                                  ...                 880                 890                 900

   LYS   ASP   ASP   GLY   SER   ALA   ASP   MET   ASN   GLN...
C A A A G A T G A C G G T T C G G C G G A T A T G A A T C A ...
               910                 920                 930 ...
                                  ...   LYS   VAL   GLU   ASN   GLY   GLU   LEU   ALA   LYS   THR
                                  ...A A A A G T C G A A A A C G G C G A G C T G G C G A A A A C
                                  ...                 940                 950                 960

   LYS   VAL   LYS   LEU   VAL   SER   ALA   SER   GLY   THR...
C A A A G T G A A A T T G G T A T C G G C A A G C G G T A C ...
               970                 980                 990 ...
                                  ...   ASN   PRO   VAL   LYS   ILE   SER   ASN   VAL   ALA   ASP
                                  ...A A A T C C G G T G A A A A T T A G C A A T G T T G C A G A
                                  ...                 1000                1010                1020

   GLY   THR   GLU   ASP   THR   ASP   ALA   VAL   SER   PHE...
C G G C A C G G A A G A C A C C G A T G C G G T C A G C T T ...
               1030                1040                1050 ...
```

FIG.19F

```
                                    ...  LYS  GLN  LEU  LYS  ALA  LEU  GLN  ASP  LYS  GLN
                                    ...T A A G C A A T T A A A A G C C T T G C A A G A C A A A C A
                                    ...            1060              1070              1080

   VAL  THR  LEU  SER  THR  SER  ASN  ALA  TYR  ALA...
G G T T A C G T T G A G C A C G A G C A A T G C T T A T G C ...
            1090              1100              1110 ...
                                    ...  ASN  GLY  GLY  THR  ASP  ASN  ASP  GLY  GLY  LYS
                                    ...C A A T G G C G G T A C A G A T A A C G A C G G C G G C A A
                                    ...            1120              1130              1140

   ALA  THR  GLN  THR  LEU  SER  ASN  GLY  LEU  ASN...
G G C A A C T C A A A C T T T A A G C A A T G G T T T G A A ...
            1150              1160              1170 ...
                                    ...  PHE  LYS  PHE  LYS  SER  SER  ASP  GLY  GLU  LEU
                                    ...T T T T A A A T T T A A A T C T A G C G A T G G C G A G T T
                                    ...            1180              1190              1200

   LEU  LYS  ILE  SER  ALA  THR  GLY  ASP  THR  VAL...
G T T G A A A A T T A G C G C G A C C G G C G A T A C G G T ...
            1210              1220              1230 ...
                                    ...  THR  PHE  THR  PRO  LYS  LYS  GLY  SER  VAL  GLN
                                    ...T A C T T T T A C G C C G A A A A A A G G T T C G G T A C A
                                    ...            1240              1250              1260
```

FIG.19G

```
   VAL  GLY  ASP  ASP  GLY  LYS  ALA  SER  ILE  SER...
G G T T G G C G A T G A T G G C A A G G C T T C A A T T T C ...
          1270              1280              1290 ...
                            ...  LYS  GLY  ALA  ASN  THR  THR  GLU  GLY  LEU  VAL
                            ...A A A A G G T G C A A A T A C A A C T G A A G G T T T G G T
                            ...           1300              1310              1320

   GLU  ALA  SER  GLU  LEU  VAL  GLU  SER  LEU  ASN...
T G A G G C T T C T G A A T T G G T T G A A A G C C T G A A ...
          1330              1340              1350 ...
                            ...  LYS  LEU  GLY  TRP  LYS  VAL  GLY  VAL  GLU  LYS
                            ...C A A A C T G G G T T G G A A A G T A G G G G T T G A G A A
                            ...           1360              1370              1380

   VAL  GLY  SER  GLY  GLU  LEU  ASP  GLY  THR  SER...
A G T C G G C A G C G G C G A G C T T G A T G G T A C A T C ...
          1390              1400              1410 ...
                            ...  LYS  GLU  THR  LEU  VAL  LYS  SER  GLY  ASP  LYS
                            ...C A A G G A A A C T T T A G T G A A G T C G G G C G A T A A
                            ...           1420              1430              1440

   VAL  THR  LEU  LYS  ALA  GLY  ASP  ASN  LEU  LYS...
A G T A A C T T T G A A A G C C G G C G A C A A T C T G A A ...
          1450              1460              1470 ...
```

FIG.19H

```
                                    ... VAL  LYS  GLN  GLU  GLY  THR  ASN  PHE  THR  TYR
                                    ...G G T C A A A C A A G A G G G C A C A A A C T T C A C T T A
                                    ...              1480              1490              1500

  ALA  LEU  LYS  ASP  GLU  LEU  THR  GLY  VAL  LYS...
C G C G C T C A A A G A T G A A T T G A C G G G C G T G A A ...
            1510              1520              1530 ...
                                    ...  SER  VAL  GLU  PHE  LYS  ASP  THR  ALA  ASN  GLY
                                    ...G A G C G T G G A G T T T A A A G A C A C G G C G A A T G G
                                    ...              1540              1550              1560

  ALA  ASN  GLY  ALA  SER  THR  LYS  ILE  THR  LYS...
T G C A A A C G G T G C A A G C A C G A A G A T T A C C A A ...
            1570              1580              1590 ...
                                    ...  ASP  GLY  LEU  THR  ILE  THR  LEU  ALA  ASN  GLY
                                    ...A G A C G G C T T G A C C A T T A C G C T G G C A A A C G G
                                    ...              1600              1610              1620

  ALA  ASN  GLY  ALA  THR  VAL  THR  ASP  ALA  ASP...
T G C G A A T G G T G C G A C G G T G A C T G A T G C C G A ...
            1630              1640              1650 ...
                                    ...  LYS  ILE  LYS  VAL  ALA  SER  ASP  GLY  ILE  SER
                                    ...C A A G A T T A A A G T T G C T T C G G A C G G C A T T A G
                                    ...              1660              1670              1680
```

FIG.19I

```
   ALA  GLY  ASN  LYS  ALA  VAL  LYS  ASN  VAL  ALA...
C G C G G G T A A T A A A G C A G T T A A A A A C G T C G C ...
          1690              1700              1710 ...
                            ...  ALA  GLY  GLU  ILE  SER  ALA  THR  SER  THR  ASP
                            ...G G C A G G C G A A A T T T C T G C C A C T T C C A C C G A
                            ...           1720              1730              1740

   ALA  ILE  ASN  GLY  SER  GLN  LEU  TYR  ALA  VAL...
T G C G A T T A A C G G A A G C C A G T T G T A T G C C G T ...
          1750              1760              1770 ...
                            ...  ALA  LYS  GLY  VAL  THR  ASN  LEU  ALA  GLY  GLN
                            ...G G C A A A A G G G G T A A C A A A C C T T G C T G G A C A
                            ...           1780              1790              1800

   VAL  ASN  ASN  LEU  GLU  GLY  LYS  VAL  ASN  LYS...
A G T G A A T A A T C T T G A G G G C A A A G T G A A T A A ...
          1810              1820              1830 ...
                            ...  VAL  GLY  LYS  ARG  ALA  ASP  ALA  GLY  THR  ALA
                            ...A G T G G G C A A A C G T G C A G A T G C A G G T A C T G C
                            ...           1840              1850              1860

   SER  ALA  LEU  ALA  ALA  SER  GLN  LEU  PRO  GLN...
A A G T G C A T T A G C G G C T T C A C A G T T A C C A C A ...
          1870              1880              1890 ...
```

FIG.19J

```
                                    ...  ALA  THR  MET  PRO  GLY  LYS  SER  MET  VAL  SER
                                    ...A G C C A C T A T G C C A G G T A A A T C A A T G G T T T C
                                    ...            1900            1910            1920

   ILE  ALA  GLY  SER  SER  TYR  GLN  GLY  GLN  ASN...
T A T T G C G G G A A G T A G T T A T C A A G G T C A A A A ...
            1930            1940            1950 ...
                                    ...  GLY  LEU  ALA  ILE  GLY  VAL  SER  ARG  ILE  SER
                                    ...T G G T T T A G C T A T C G G G G T A T C A A G A A T T T C
                                    ...            1960            1970            1980

   ASP  ASN  GLY  LYS  VAL  ILE  ILE  ARG  LEU  SER...
C G A T A A T G G C A A A G T G A T T A T T C G C T T G T C ...
            1990            2000            2010 ...
                                    ...  GLY  THR  THR  ASN  SER  GLN  GLY  LYS  THR  GLY
                                    ...A G G C A C A A C C A A T A G T C A A G G T A A A A C A G G
                                    ...            2020            2030            2040

   VAL  ALA  ALA  GLY  VAL  GLY  TYR  GLN  TRP  ***
C G T T G C A G C A G G T G T T G G T T A C C A G T G G T A ...

                                    ...A T A G A A T T C
```

FIG.20A

NTHi strain 29 Hia

```
                                        MET  ASN  LYS ...
T T A A A T A T A A G G T A A A T A A A A A T G A A C A A A...
                10                  20                  30...
                                ... ILE  PHE  ASN  VAL  ILE  TRP  ASN  VAL  VAL  THR
                                ... A T T T T T A A C G T T A T T T G G A A T G T T G T G A C T
                                ...                 40                  50                  60

 GLN  THR  TRP  VAL  VAL  VAL  SER  GLU  LEU  THR ...
C A A A C T T G G G T T G T C G T A T C T G A A C T C A C T...
                70                  80                  90...
                                ... ARG  ALA  HIS  THR  LYS  CYS  ALA  SER  ALA  THR
                                ... C G C G C C C A C A C C A A A T G C G C C T C C G C C A C C
                                ...                 100                 110                 120

 VAL  ALA  VAL  ALA  VAL  LEU  ALA  THR  ALA  LEU ...
G T G G C G G T T G C C C G T A T T G G C A A C T G C G T T G...
                130                 140                 150...
                                ... SER  ALA  THR  ALA  GLU  ALA  ASN  ASN  ASN  THR
                                ... T C T G C A A C G G C T G A A G C G A A C A A C A A T A C T
                                ...                 160                 170                 180

 SER  VAL  THR  ASN  GLY  LEU  ASN  ALA  TYR  GLY ...
T C T G T T A C G A A T G G G T T G A A T G C T T A T G G C...
                190                 200                 210...
```

FIG.20B

```
                                    ... ASP  THR  ASN  PHE  ASN  THR  THR  ASN  ASN  SER
                                    ... G A T A C T A A T T T T A A T A C A A C C A A T A A T T C G
                                    ...                220                230                240

 ILE  ALA  ASP  LEU  GLU  LYS  HIS  VAL  GLN  ASP ...
A T A G C A G A T T T G G A A A A A C A C G T T C A A G A T...
               250                260                270...
                                    ... ALA  TYR  LYS  GLY  LEU  LEU  ASN  LEU  ASN  GLU
                                    ... G C T T A T A A A G G C T T A T T A A A T C T G A A T G A A
                                    ...                280                290                300

 LYS  ASP  THR  ASN  LYS  SER  SER  PHE  LEU  VAL ...
A A A G A T A C A A A T A A G T C A A G T T T C T T G G T T...
               310                320                330...
                                    ... ALA  ASP  ASN  THR  ALA  ALA  THR  VAL  GLY  ASN
                                    ... G C C G A C A A T A C C G C C G C A A C C G T A G G C A A T
                                    ...                340                350                360

 LEU  ARG  LYS  LEU  GLY  TRP  VAL  LEU  SER  SER ...
T T G C G T A A A T T G G G C T G G G T A T T G T C T A G C...
               370                380                390...
                                    ... LYS  ASN  GLY  THR  ARG  ASN  GLU  LYS  SER  TYR
                                    ... A A A A A C G G C A C A A G G A A C G A G A A A A G C T A T
                                    ...                400                410                420
```

FIG.20C

```
 GLN  VAL  LYS  GLN  ALA  ASP  GLU  VAL  LEU  PHE ...
C A A G T A A A A C A A G C T G A T G A A G T T C T C T T T...
              430                  440                  450...
                                  ... THR  GLY  SER  GLY  ALA  ALA  THR  VAL  SER  SER
                                  ... A C T G G A T C T G G T G C T G C A A C G G T T A G T T C C
                                  ...                  460                  470                  480

 SER  SER  LYS  ASP  GLY  LYS  HIS  THR  ILE  THR ...
A G C T C T A A A G A C G G T A A A C A T A C C A T T A C C...
              490                  500                  510...
                                  ... ILE  SER  VAL  THR  LYS  GLY  SER  PHE  ALA  GLU
                                  ... A T T T C T G T T A C C A A A G G T A G T T T T G C T G A G
                                  ...                  520                  530                  540

 VAL  LYS  THR  ASP  ALA  THR  THR  GLY  GLY  GLN ...
G T A A A A A C T G A T G C A A C T A C T G G A G G T C A A...
              550                  560                  570...
                                  ... VAL  ASN  ALA  ASP  ARG  GLY  LYS  VAL  LYS  ALA
                                  ... G T A A A C G C C G A C C G T G G T A A A G T G A A A G C T
                                  ...                  580                  590                  600

 GLU  ASP  GLU  ASN  GLY  ALA  ASP  VAL  ASP  LYS ...
G A G G A C G A G A A T G G A G C T G A T G T T G A T A A G...
              610                  620                  630...
```

FIG.20D

```
                                        ... LYS  VAL  ALA  THR  VAL  LYS  ASP  VAL  ALA  LYS
                                        ... A A A G T T G C A A C T G T A A A A G A T G T T G C T A A G
                                        ...              640                  650                  660

 ALA  ILE  ASN  ASP  ALA  ALA  THR  PHE  VAL  LYS ...
G C G A T T A A C G A T G C C G C A A C T T T C G T G A A A...
              670                  680                  690...
                                        ... VAL  GLU  SER  THR  ASP  ASP  ASP  ILE  GLU  ASN
                                        ... G T G G A A A G C A C A G A T G A T G A C A T T G A A A A T
                                        ...              700                  710                  720

 GLY  ALA  ALA  GLY  LYS  ASN  GLU  THR  THR  ASP ...
G G T G C T G C A G G C A A A A A T G A A A C T A C A G A C...
              730                  740                  750...
                                        ... GLN  ALA  LEU  LYS  ALA  GLY  ASP  THR  LEU  THR
                                        ... C A A G C T C T C A A A G C A G G C G A C A C C T T A A C C
                                        ...              760                  770                  780

 LEU  LYS  ALA  GLY  LYS  ASN  LEU  LYS  ALA  LYS ...
T T A A A A G C G G G T A A A A A C T T A A A A G C T A A G...
              790                  800                  810...
                                        ... LEU  ASP  GLN  ASN  GLY  LYS  SER  VAL  THR  PHE
                                        ... T T A G A C C A A A A T G G T A A A T C A G T A A C C T T T
                                        ...              820                  830                  840
```

FIG.20E

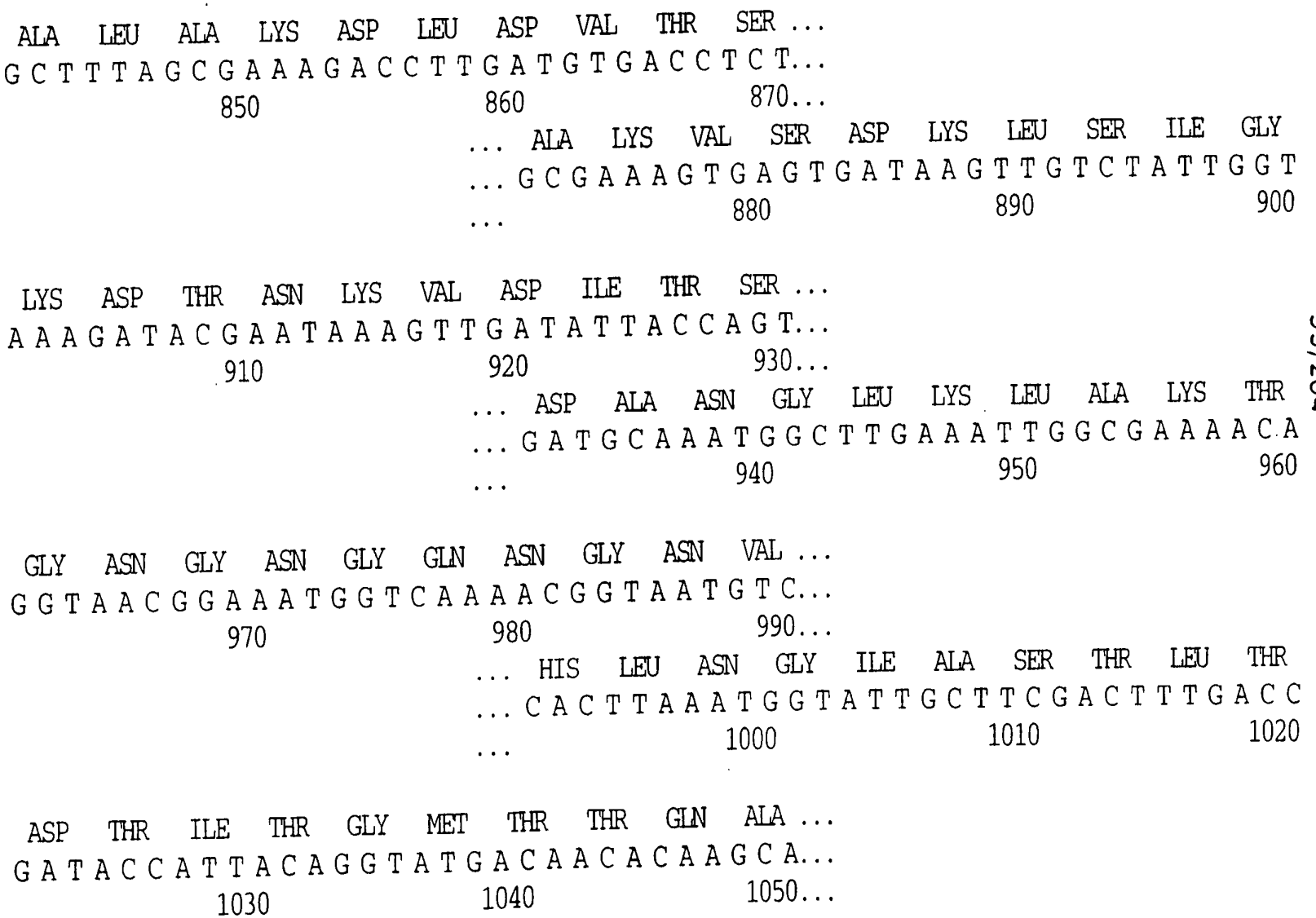


FIG.20F

```
                                    ... SER ASN GLY VAL ALA VAL GLN ASN HIS ASN
                                    ... A G C A A T G G C G T G G C T G T G C A G A A T C A T A A T
                                    ...          1060          1070          1080

 ARG ALA ALA SER VAL ALA ASP VAL LEU ASN ...
C G T G C T G C G A G T G T G G C T G A T G T A T T A A A T...
          1090          1100          1110...
                                    ... ALA GLY TRP ASN ILE GLN GLY ASN GLY ALA
                                    ... G C A G G C T G G A A T A T T C A A G G C A A C G G A G C G
                                    ...          1120          1130          1140

 SER VAL ASP PHE VAL ASN ALA TYR ASP THR ...
A G C G T T G A T T T T G T C A A T G C T T A C G A C A C A...
          1150          1160          1170...
                                    ... VAL ASP PHE VAL ASN GLY THR ASN THR ASN
                                    ... G T A G A T T T T G T C A A T G G T A C A A A C A C C A A T
                                    ...          1180          1190          1200

 VAL ASN VAL THR THR ASP THR ALA HIS LYS ...
G T G A A C G T T A C G A C T G A T A C G G C T C A C A A A...
          1210          1220          1230...
                                    ... LYS THR THR VAL ARG VAL ASP VAL THR GLY
                                    ... A A G A C A A C C G T C C G T G T G G A T G T A A C A G G C
                                    ...          1240          1250          1260
```

FIG.20G

```
 LEU  PRO  VAL  GLN  TYR  VAL  THR  GLU  ASP  GLY ...
T T G C C G G T T C A A T A T G T T A C G G A A G A C G G C...
          1270              1280              1290...
                            ... LYS  THR  VAL  VAL  LYS  VAL  ASP  ASN  LYS  TYR
                            ... A A A A C C G T T G T G A A A G T G G A C A A T A A G T A T
                            ...            1300              1310              1320

 TYR  GLU  ALA  LYS  GLN  ASP  GLY  SER  ALA  ASP ...
T A C G A A G C T A A G C A A G A C G G T T C G G C G G A T...
          1330              1340              1350...
                            ... MET  ASP  LYS  LYS  VAL  GLU  ASN  GLY  GLU  LEU
                            ... A T G G A T A A A A A A G T C G A A A A T G G C G A G C T G
                            ...            1360              1370              1380

 ALA  LYS  THR  LYS  VAL  LYS  LEU  VAL  SER  ALA ...
G C G A A A A C C A A A G T G A A A T T G G T G T C G G C A...
          1390              1400              1410...
                            ... SER  GLY  GLN  ASN  PRO  VAL  LYS  ILE  SER  ASN
                            ... A G C G G T C A A A A T C C G G T G A A A A T C A G C A A T
                            ...            1420              1430              1440

 VAL  ALA  GLU  GLY  THR  GLU  GLU  ASN  ASP  ALA ...
G T T G C G G A A G G C A C G G A A G A A A A C G A T G C G...
          1450              1460              1470...
```

FIG.20H

```
... VAL  SER  PHE  LYS  GLN  LEU  LYS  ALA  LEU  GLN
...GTCAGCTTTAAGCAATTGAAAGCCTTGCAA
...          1480          1490          1500

 GLU  LYS  GLN  VAL  THR  LEU  THR  ALA  SER  ASN ...
GAGAAACAGGTTACTTTAACTGCGAGCAAT...
          1510          1520          1530...
                ... ALA  TYR  ALA  ASN  GLY  GLY  ASN  ASP  ALA  ASP
                ...GCTTATGCCAATGGTGGTAACGATGCCGAC
                ...          1540          1550          1560

 GLY  GLY  LYS  ALA  THR  GLN  THR  LEU  ASN  ASN ...
GGCGGCAAGGCAACTCAAACTTTAAACAAT...
          1570          1580          1590...
                ... GLY  LEU  ASN  PHE  LYS  PHE  LYS  SER  THR  ASP
                ...GGTTTGAATTTTAAATTTAAATCCACAGAC
                ...          1600          1610          1620

 GLY  GLU  LEU  LEU  ASN  ILE  LYS  VAL  GLU  ASN ...
GGCGAGTTGTTGAACATCAAAGTAGAAAAT...
          1630          1640          1650...
                ... ASP  THR  VAL  THR  PHE  THR  PRO  LYS  LYS  GLY
                ...GACACAGTTACCTTTACGCCGAAAAAAGGT
                ...          1660          1670          1680
```

FIG.20I

```
 SER  VAL  GLN  VAL  GLY  GLU  ASP  GLY  LYS  ALA ...
T C G G T A C A G G T T G G C G A A G A C G G T A A G G C T...
          1690              1700              1710...
                                ... THR  ILE  GLN  ASN  GLY  THR  LYS  THR  THR  ASP
                                ... A C G A T T C A A A A T G G T A C G A A A A C A A C C G A C
                                ...           1720              1730              1740

 GLY  LEU  VAL  GLU  ALA  SER  GLU  LEU  VAL  GLU ...
G G T T T G G T T G A A G C T T C C G A A T T G G T T G A A...
          1750              1760              1770...
                                ... SER  LEU  ASN  LYS  LEU  GLY  TRP  LYS  VAL  GLY
                                ... A G C C T G A A C A A A C T G G G C T G G A A A G T G G G C
                                ...           1780              1790              1800

 VAL  ASP  LYS  ASP  GLY  SER  GLY  GLU  LEU  ASP ...
G T T G A T A A A G A C G G C A G C G G C G A G C T T G A T...
          1810              1820              1830...
                                ... GLY  ALA  SER  ASN  GLU  THR  LEU  VAL  LYS  SER
                                ... G G T G C A T C C A A T G A A A C T T T A G T G A A G T C G
                                ...           1840              1850              1860

 GLY  ASP  LYS  VAL  THR  LEU  LYS  ALA  GLY  GLU ...
G G C G A T A A A G T A A C T T T G A A A G C C G G C G A G...
          1870              1880              1890...
```

FIG.20J

```
                                    ... ASN  LEU  LYS  VAL  LYS  GLN  ASP  GLY  THR  ASN
                                    ... A A T C T G A A G G T C A A A C A A G A C G G C A C A A A C
                                    ...              1900              1910              1920

 PHE  THR  TYR  ALA  LEU  LYS  ASP  GLU  LEU  THR ...
T T C A C T T A C G C G C T C A A A G A T G A A T T G A C G...
              1930              1940              1950...
                                    ... GLY  VAL  LYS  SER  VAL  GLU  PHE  LYS  ASP  THR
                                    ... G G C G T G A A G A G C G T G G A G T T T A A A G A C A C G
                                    ...              1960              1970              1980

 ALA  ASN  GLY  SER  ASN  GLY  ALA  SER  THR  LYS ...
G C G A A T G G T T C A A A C G G T G C A A G C A C G A A G...
              1990              2000              2010...
                                    ... ILE  THR  LYS  ASP  GLY  LEU  THR  ILE  THR  SER
                                    ... A T T A C C A A A G A C G G C T T G A C C A T T A C G T C G
                                    ...              2020              2030              2040

 ALA  ASN  GLY  ALA  ASN  GLY  ALA  ALA  ALA  THR ...
G C A A A C G G T G C G A A T G G T G C G G C G G C G A C T...
              2050              2060              2070...
                                    ... ASP  ALA  ASP  LYS  ILE  LYS  VAL  ALA  SER  ASP
                                    ... G A T G C G G A C A A G A T T A A A G T G G C T T C A G A C
                                    ...              2080              2090              2100
```

FIG.20K

```
 GLY  ILE  SER  ALA  GLY  ASN  LYS  ALA  VAL  LYS ...
G G C A T C A G T G C G G G T A A T A A A G C G G T T A A A...
            2110              2120              2130...
                              ... ASN  VAL  VAL  SER  GLY  LEU  LYS  LYS  PHE  GLY
                              ... A A C G T T G T G A G C G G A C T G A A G A A A T T T G G T
                              ...              2140              2150              2160

 ASP  ALA  ASN  PHE  ASN  PRO  LEU  THR  SER  SER ...
G A T G C G A A T T T C A A T C C A C T G A C C A G T T C C...
            2170              2180              2190...
                              ... ALA  ASP  ASN  LEU  THR  LYS  GLN  TYR  ASP  ASP
                              ... G C C G A C A A C T T A A C G A A A C A A T A T G A C G A T
                              ...              2200              2210              2220

 ALA  TYR  LYS  GLY  LEU  THR  ASN  LEU  ASP  GLU ...
G C C T A T A A A G G C T T G A C C A A T T T G G A T G A A...
            2230              2240              2250...
                              ... LYS  GLY  ALA  ASP  LYS  GLN  THR  LEU  THR  VAL
                              ... A A A G G T G C G G A C A A G C A A A C T C T G A C T G T T
                              ...              2260              2270              2280

 ALA  ASP  ASN  THR  ALA  ALA  THR  VAL  GLY  ASP ...
G C C G A C A A T A C T G C C G C A A C C G T G G G C G A T...
            2290              2300              2310...
```

FIG.20L

```
                                    ... LEU  ARG  GLY  LEU  GLY  TRP  VAL  ILE  SER  ALA
                                    ... T T G C G C G G C T T G G G C T G G G T C A T T T C T G C G
                                    ...              2320              2330              2340

 ASP  LYS  THR  THR  GLY  GLU  LEU  ASN  LYS  GLU ...
G A C A A A A C C A C A G G C G A A C T C A A T A A G G A A...
             2350              2360              2370...
                                    ... TYR  ASN  ALA  GLN  VAL  ARG  ASN  ALA  ASN  GLU
                                    ... T A C A A C G C G C A A G T G C G T A A C G C C A A T G A A
                                    ...              2380              2390              2400

 VAL  LYS  PHE  LYS  SER  GLY  ASN  GLY  ILE  HIS ...
G T G A A A T T C A A G A G C G G C A A C G G T A T C C A T...
             2410              2420              2430...
                                    ... VAL  SER  GLY  LYS  THR  VAL  ASN  GLY  ARG  ARG
                                    ... G T T T C C G G T A A A A C G G T C A A C G G T A G G C G C
                                    ...              2440              2450              2460

 GLU  ILE  THR  PHE  GLU  LEU  ALA  LYS  ASP  GLU ...
G A A A T T A C T T T T G A A T T G G C T A A A G A C G A A...
             2470              2480              2490...
                                    ... ASN  ALA  ILE  ALA  PHE  GLY  TYR  GLY  SER  LYS
                                    ... A A T G C C A T T G C T T T C G G T T A T G G C T C A A A A
                                    ...              2500              2510              2520
```

FIG.20M

```
 ALA  LEU  ARG  ASP  ASN  THR  VAL  ALA  ILE  GLY ...
G C C T T G C G C G A T A A C A C G G T G G C A A T T G G T...
            2530              2540              2550...
                              ... THR  GLY  ASN  VAL  VAL  ASN  ALA  GLU  LYS  SER
                              ... A C G G G C A A C G T T G T G A A T G C G G A A A A A T C T
                              ...            2560              2570              2580

 GLY  ALA  PHE  GLY  ASP  PRO  ASN  TYR  ILE  GLU ...
G G T G C A T T C G G C G A T C C G A A C T A C A T C G A A...
            2590              2600              2610...
                              ... ASP  LYS  ALA  GLY  GLY  SER  TYR  ALA  PHE  GLY
                              ... G A T A A A G C C G G T G G C A G C T A C G C T T T C G G T
                              ...            2620              2630              2640

 ASN  ASP  ASN  ARG  ILE  THR  SER  LYS  ASN  THR ...
A A C G A T A A C C G T A T T A C T T C T A A A A A C A C T...
            2650              2660              2670...
                              ... PHE  VAL  LEU  GLY  ASN  GLY  VAL  ASN  ALA  LYS
                              ... T T T G T G T T G G G T A A T G G A G T T A A T G C G A A A
                              ...            2680              2690              2700

 TYR  LYS  ALA  ASN  GLY  ASP  VAL  ASP  THR  GLU ...
T A T A A A G C C A A T G G A G A T G T T G A T A C G G A A...
            2710              2720              2730...
```

FIG.20N

```
                                    ... THR  VAL  THR  VAL  LYS  ASP  LYS  ASP  GLY  LYS
                                    ... A C C G T A A C C G T T A A G G A C A A A G A C G G T A A A
                                    ...              2740              2750              2760

 GLU  THR  THR  VAL  THR  VAL  PRO  LYS  ALA  LEU ...
G A G A C T A C C G T T A C T G T T C C T A A A G C G T T A...
              2770              2780              2790...
                                    ... GLY  ALA  THR  VAL  GLU  ASN  SER  VAL  TYR  LEU
                                    ... G G G G C T A C G G T T G A A A A C T C C G T T T A T T T G
                                    ...              2800              2810              2820

 GLY  ASN  LYS  SER  THR  ALA  THR  LYS  ASP  LYS ...
G G T A A T A A A T C G A C T G C G A C A A A A G A T A A G...
              2830              2840              2850...
                                    ... GLY  LYS  ASN  LEU  LYS  SER  ASP  GLY  THR  ALA
                                    ... G G T A A A A A C C T G A A A T C T G A T G G T A C G G C G
                                    ...              2860              2870              2880

 GLY  ASN  THR  THR  THR  ALA  GLY  THR  THR  GLY ...
G G T A A C A C T A C A A C T G C T G G C A C A A C G G G T...
              2890              2900              2910...
                                    ... THR  VAL  ASN  GLY  PHE  ALA  GLY  ALA  THR  ALA
                                    ... A C G G T A A A C G G C T T T G C C G G T G C A A C G G C G
                                    ...              2920              2930              2940
```

FIG.20O

```
 HIS  GLY  ALA  VAL  SER  VAL  GLY  ALA  SER  GLY ...
C A C G G T G C G G T T T C T G T C G G C G C A A G C G G C...
            2950              2960              2970...
                              ... GLU  GLU  ARG  ARG  ILE  GLN  ASN  VAL  ALA  ALA
                              ... G A A G A A A G A C G T A T C C A A A A C G T C G C G G C A
                              ...            2980              2990              3000

 GLY  GLU  ILE  SER  ALA  THR  SER  THR  ASP  ALA ...
G G C G A A A T T T C C G C C A C T T C C A C C G A T G C G...
            3010              3020              3030...
                              ... ILE  ASN  GLY  SER  GLN  LEU  TYR  ALA  VAL  ALA
                              ... A T T A A C G G C A G C C A G T T G T A T G C T G T G G C A
                              ...            3040              3050              3060

 LYS  GLY  VAL  THR  ASN  LEU  ALA  GLY  GLN  VAL ...
A A A G G G G T A A C A A A T C T T G C T G G A C A A G T G...
            3070              3080              3090...
                              ... ASN  LYS  VAL  GLY  LYS  ARG  ALA  ASP  ALA  GLY
                              ... A A T A A A G T G G G C A A A C G T G C A G A T G C A G G T
                              ...            3100              3110              3120

 THR  ALA  SER  ALA  LEU  ALA  ALA  SER  GLN  LEU ...
A C A G C A A G T G C A T T A G C A G C T T C A C A G T T A...
            3130              3140              3150...
```

FIG.20P

```
                                  ... PRO  GLN  ALA  SER  MET  PRO  GLY  LYS  SER  MET
                                  ... C C A C A A G C C T C T A T G C C A G G T A A A T C A A T G
                                  ...              3160              3170              3180

 VAL  SER  ILE  ALA  GLY  SER  SER  TYR  GLN  GLY ...
G T T T C T A T T G C G G G A A G T A G T T A T C A A G G T...
            3190              3200              3210...
                                  ... GLN  ASN  GLY  LEU  ALA  ILE  GLY  VAL  SER  ARG
                                  ... C A A A A T G G T T T A G C T A T C G G G G T A T C A C G A
                                  ...              3220              3230              3240

 ILE  SER  ASP  ASN  GLY  LYS  VAL  ILE  ILE  ARG ...
A T T T C C G A T A A T G G C A A A G T G A T T A T T C G C...
            3250              3260              3270...
                                  ... LEU  SER  GLY  THR  THR  ASN  SER  GLN  GLY  LYS
                                  ... T T G T C A G G C A C A A C C A A T A G C C A A G G T A A A
                                  ...              3280              3290              3300

 THR  GLY  VAL  ALA  ALA  GLY  VAL  GLY  TYR  GLN ...
A C A G G C G T T G C A G C A G G T G T T G G T T A C C A G...
            3310              3320              3330...
                                  ... TRP  ***
                                  ... T G G T A A T A G A A T T C C G G A T C C G C
                                  ...              3340              3350
```

FIG.21A

NTHi strain M4071 Hia

```
                    MET  ASN  LYS  ILE  PHE  ASN  VAL...
G C G A A T T C A T A T G A A C A A A A T T T T T A A C G T ...
              10                  20                  30 ...
                                ...  ILE  TRP  ASN  VAL  MET  THR  GLN  THR  TRP  ALA
                                ...T A T T T G G A A T G T T A T G A C T C A A A C T T G G G C
                                ...              40                  50                  60

  VAL  VAL  SER  GLU  LEU  THR  ARG  ALA  HIS  THR...
T G T C G T A T C T G A A C T C A C T C G C G C C C A C A C ...
              70                  80                  90 ...
                                ...  LYS  ARG  ALA  SER  ALA  THR  VAL  ALA  THR  ALA
                                ...C A A A C G T G C C T C C G C A A C C G T G G C A A C C G C
                                ...              100                 110                 120

  VAL  LEU  ALA  THR  LEU  LEU  SER  THR  THR  VAL...
C G T A T T G G C G A C G T T G T T G T C T A C A A C A G T ...
              130                 140                 150 ...
                                ...  GLN  ALA  THR  THR  THR  GLY  GLY  THR  THR  SER
                                ...T C A G G C G A C A A C T A C T G G C G G T A C G A C A A G
                                ...              160                 170                 180

  THR  ASN  GLY  LEU  LYS  ALA  TYR  GLY  SER  THR...
T A C A A A C G G T T T G A A A G C T T A T G G A A G T A C ...
              190                 200                 210 ...
```

FIG.21B

```
                                    ...  ASN  ASN  PRO  ASN  PHE  ASN  ALA  ALA  GLY  ASN
                                    ...G A A T A A T C C G A A T T T C A A T G C T G C A G G T A A
                                    ...              220              230              240

  SER  ALA  THR  ASP  LEU  ALA  ARG  GLN  PHE  ASP...
C T C T G C A A C T G A T T T A G C T A G A C A G T T T G A ...
             250              260              270 ...
                                    ...  GLY  ALA  TYR  ASP  GLY  LEU  LEU  ASN  LEU  ASN
                                    ...T G G T G C T T A T G A C G G T T T A T T A A A T C T A A A
                                    ...              280              290              300

  GLU  LYS  ASP  ALA  ASN  LYS  ASN  LEU  LEU  VAL...
T G A A A A A G A T G C G A A T A A A A A T C T G T T G G T ...
             310              320              330 ...
                                    ...  THR  ASP  ASP  LYS  ALA  ALA  THR  VAL  GLY  ASN
                                    ...G A C T G A T G A T A A G G C G G C G A C C G T A G G C A A
                                    ...              340              350              360

  LEU  ARG  LYS  LEU  GLY  TRP  VAL  LEU  SER  SER...
T T T G C G T A A A T T G G G T T G G G T A T T G T C T A G ...
             370              380              390 ...
                                    ...  LYS  ASN  GLY  THR  ARG  ASN  GLU  LYS  SER  GLN
                                    ...T A A A A A C G G C A C A A G G A A C G A G A A A A G C C A
                                    ...              400              410              420
```

FIG.21C

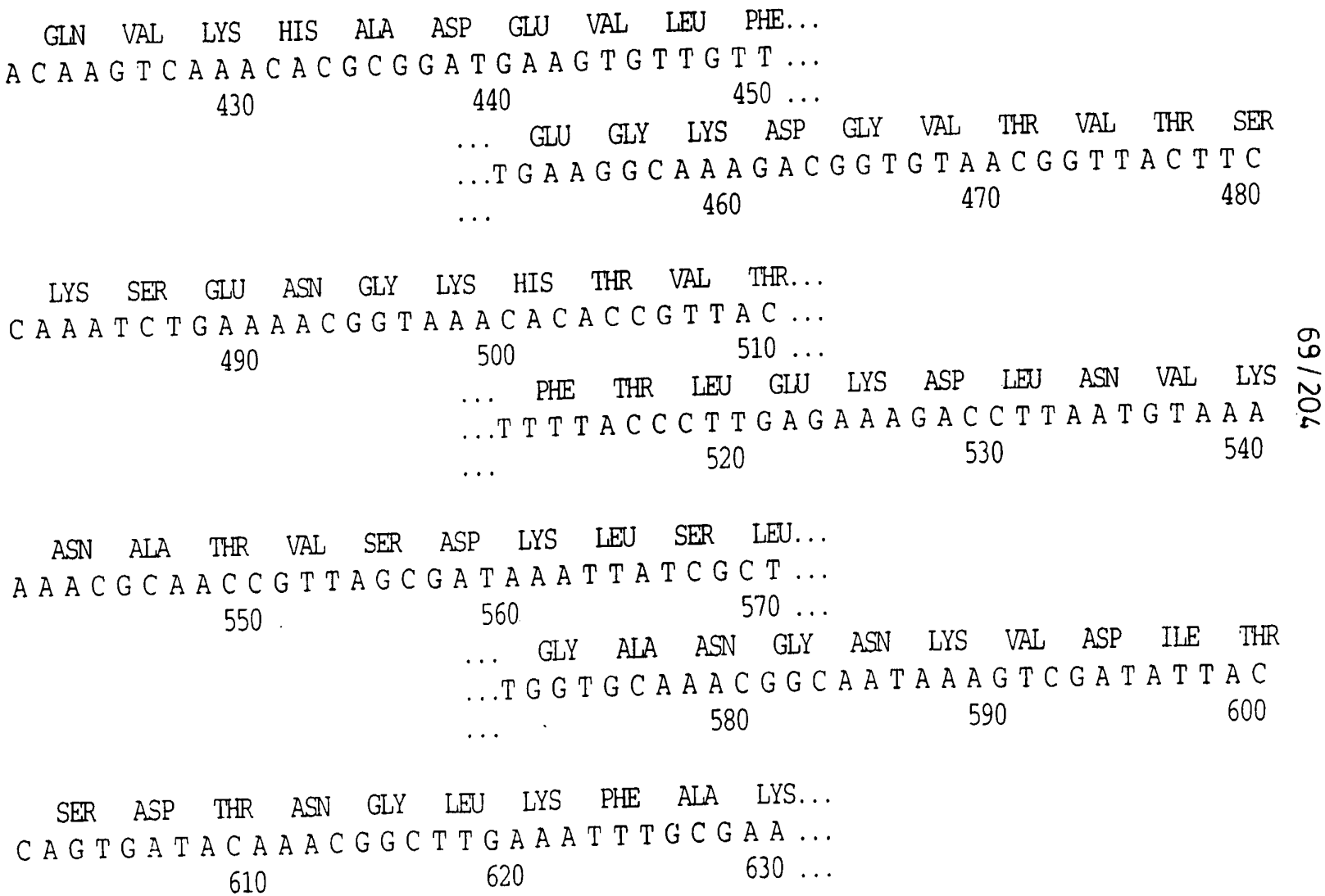


FIG.21D

```
                                    ...  PRO  SER  THR  ASN  GLY  GLN  ASN  GLY  ASN  VAL
                                    ...A C C A A G T A C G A A T G G T C A A A A C G G T A A T G T
                                    ...              640                  650                  660

  HIS  LEU  ASN  GLY  ILE  ALA  SER  THR  LEU  THR...
T C A C T T A A A C G G T A T T G C C T C T A C C T T A A C ...
              670                  680                  690 ...
                                    ...  ASP  THR  ILE  THR  GLY  THR  THR  LYS  SER  ALA
                                    ...T G A C A C A A T T A C A G G T A C A A C A A A A T C T G C
                                    ...              700                  710                  720

  THR  ASN  GLY  VAL  ASP  VAL  GLN  ASN  HIS  ASN...
A A C T A A T G G T G T A G A T G T G C A G A A T C A T A A ...
              730                  740                  750 ...
                                    ...  ARG  ALA  ALA  SER  VAL  ALA  ASP  VAL  LEU  ASN
                                    ...T C G T G C T G C G A G T G T A G C T G A T G T A T T G A A
                                    ...              760                  770                  780

  ALA  GLY  TRP  ASN  ILE  GLN  GLY  ASN  GLY  ALA...
T G C A G G C T G G A A T A T T C A A G G C A A C G G A G C ...
              790                  800                  810 ...
                                    ...  SER  VAL  ASP  PHE  VAL  ASN  THR  TYR  ASP  THR
                                    ...G A G C G T T G A T T T T G T C A A T A C T T A C G A C A C
                                    ...              820                  830                  840
```

FIG.21E

```
   VAL  ASP  PHE  VAL  ASN  GLY  LEU  ASN  THR  ASN...
A G T A G A T T T T G T C A A T G G T T T A A A T A C C A A ...
            850                 860                 870 ...
                                ...  VAL  ASN  VAL  THR  THR  ASP  THR  ALA  HIS  ASN
                                ...T G T G A A C G T T A C G A C T G A T A C G G C T C A C A A
                                ...                 880                 890                 900

   LYS  LYS  THR  THR  VAL  ARG  VAL  ASP  VAL  THR...
C A A A A A G A C A A C C G T C C G T G T G G A T G T A A C ...
            910                 920                 930 ...
                                ...  GLY  LEU  PRO  VAL  GLN  TYR  VAL  THR  GLU  ASP
                                ...G G G C T T G C C G G T C C A A T A T G T T A C G G A A G A
                                ...                 940                 950                 960

   GLY  GLU  THR  VAL  VAL  LYS  VAL  GLY  ASN  GLU...
C G G C G A A A C C G T T G T G A A A G T G G G C A A T G A ...
            970                 980                 990 ...
                                ...  TYR  TYR  GLU  ALA  LYS  GLN  ASP  GLY  SER  ALA
                                ...G T A T T A C G A A G C C A A G C A A G A C G G T T C G G C
                                ...                 1000                1010                1020

   ASP  MET  ASP  LYS  LYS  VAL  GLU  ASN  GLY  LYS...
G G A T A T G G A T A A A A A A G T C G A A A A T G G C A A ...
            1030                1040                1050 ...
```

FIG.21F

```
                                    ...  LEU  ALA  LYS  THR  LYS  VAL  LYS  LEU  VAL  SER
                                    ...G C T G G C G A A A A C T A A A G T T A A A T T G G T A T C
                                    ...              1060              1070              1080

  ALA  ASN  GLY  THR  ASN  PRO  VAL  LYS  ILE  SER...
G G C A A A C G G T A C A A A T C C G G T G A A A A T C A G ...
            1090              1100              1110 ...
                                    ...  ASN  VAL  ALA  ASP  GLY  THR  GLU  ASN  THR  ASP
                                    ...C A A T G T T G C G G A C G G C A C G G A A A A T A C C G A
                                    ...              1120              1130              1140

  ALA  VAL  SER  PHE  LYS  GLN  LEU  LYS  ALA  LEU...
T G C G G T C A G C T T T A A G C A G T T G A A A G C C T T ...
            1150              1160              1170 ...
                                    ...  GLN  ASP  LYS  GLN  VAL  THR  LEU  SER  ALA  SER
                                    ...G C A A G A C A A A C A G G T T A C G T T A A G T G C G A G
                                    ...              1180              1190              1200

  ASN  ALA  TYR  ALA  ASN  GLY  GLY  SER  ASP  ALA...
C A A T G C T T A T G C C A A T G G C G G T A G C G A T G C ...
            1210              1220              1230 ...
                                    ...  ASP  GLY  GLY  LYS  GLY  ILE  GLN  THR  LEU  SER
                                    ...C G A C G G C G G C A A G G G A A T T C A A A C T T T A A G
                                    ...              1240              1250              1260
```

FIG.21G

```
   ASN  GLY  LEU  ASN  PHE  LYS  PHE  LYS  SER  THR...
C A A T G G T T T G A A T T T T A A A T T T A A A T C C A C ...
            1270              1280              1290 ...
                              ...  ASP  GLY  GLU  LEU  LEU  ASN  ILE  LYS  ALA  GLU
                              ...A G A C G G C G A G T T G T T G A A T A T C A A A G C A G A
                              ...            1300              1310              1320

   ASN  ASP  THR  VAL  THR  PHE  THR  PRO  LYS  LYS...
A A A T G A C A C G G T T A C C T T T A C G C C G A A A A A ...
            1330              1340              1350 ...
                              ...  GLY  SER  VAL  GLN  VAL  GLY  ASP  ASP  GLY  LYS
                              ...A G G T T C G G T G C A G G T T G G C G A T G A T G G T A A
                              ...            1360              1370              1380

   ALA  THR  ILE  GLN  ASP  GLY  ALA  LYS  THR  THR...
G G C T A C G A T T C A A G A C G G C G C A A A A A C A A C ...
            1390              1400              1410 ...
                              ...  THR  GLY  LEU  VAL  GLU  ALA  SER  GLU  LEU  VAL
                              ...T A C C G G T T T G G T T G A G G C T T C T G A A T T G G T
                              ...            1420              1430              1440

   ASP  SER  LEU  ASN  LYS  LEU  GLY  TRP  LYS  VAL...
T G A C A G C C T G A A C A A A T T G G G T T G G A A A G T ...
            1450              1460              1470 ...
```

FIG.21H

```
                                    ...  GLY  THR  GLY  THR  ASP  GLY  THR  GLY  VAL  THR
                                    ...G G G C A C C G G C A C T G A C G G C A C A G G A G T G A C
                                    ...            1480            1490            1500

  ASP  GLY  THR  HIS  THR  ASP  THR  LEU  VAL  LYS...
C G A T G G C A C G C A T A C C G A C A C T T T A G T G A A ...
            1510            1520            1530 ...
                                    ...  SER  GLY  ASP  LYS  VAL  THR  LEU  LYS  ALA  GLY
                                    ...G T C G G G C G A T A A A G T A A C T T T G A A A G C C G G
                                    ...            1540            1550            1560

  ASP  ASN  LEU  LYS  VAL  LYS  GLN  GLU  GLY  THR...
C G A C A A T C T G A A G G T C A A A C A A G A G G G T A C ...
            1570            1580            1590 ...
                                    ...  ASN  PHE  THR  TYR  ALA  LEU  LYS  ASP  GLU  LEU
                                    ...A A A C T T C A C T T A T G C G C T C A A A G A T G A A T T
                                    ...            1600            1610            1620

  THR  ASP  VAL  LYS  SER  VAL  GLU  PHE  LYS  ASP...
G A C G G A C G T G A A G A G C G T G G A G T T T A A A G A ...
            1630            1640            1650 ...
                                    ...  THR  ALA  ASN  GLY  ALA  ASN  GLY  ALA  SER  THR
                                    ...C A C G G C G A A T G G T G C A A A C G G T G C A A G C A C
                                    ...            1660            1670            1680
```

FIG.21I

```
   LYS  ILE  THR  LYS  ASP  GLY  LEU  THR  ILE  THR...
G A A G A T T A C C A A A G A C G G C T T G A C C A T T A C ...
            1690              1700              1710 ...
                              ...  PRO  ALA  ASN  GLY  ALA  GLY  ALA  ALA  GLY  ALA
                              ...G C C G G C A A A C G G T G C G G G T G C G G C A G G T G C
                              ...           1720              1730              1740
.

   ASN  THR  ALA  ASN  THR  ILE  SER  VAL  THR  LYS...
A A A C A C T G C A A A C A C C A T T A G C G T A A C C A A ...
            1750              1760              1770 ...
                              ...  ASP  GLY  ILE  SER  ALA  GLY  ASN  LYS  ALA  VAL
                              ...A G A C G G C A T T A G C G C G G G T A A T A A A G C A G T
                              ...           1780              1790              1800

   LYS  ASN  VAL  VAL  SER  GLY  LEU  LYS  LYS  PHE...
T A A A A A C G T T G T G A G C G G A C T G A A G A A A T T ...
            1810              1820              1830 ...
                              ...  GLY  ASP  ALA  ASN  PHE  ASP  PRO  LEU  THR  SER
                              ...T G G T G A T G C G A A T T T C G A T C C G C T G A C T A G
                              ...           1840              1850              1860

   SER  ALA  ASP  ASN  LEU  THR  LYS  GLN  TYR  ASP...
C T C A G C C G A C A A C T T A A C G A A A C A A T A T G A ...
            1870              1880              1890 ...
```

FIG.21J

```
                                    ...  ASN  ALA  TYR  LYS  GLY  LEU  THR  ASN  LEU  ASP
                                    ...C A A T G C C T A T A A A G G C T T G A C C A A T C T G G A
                                    ...              1900              1910              1920

  GLU  LYS  SER  LYS  GLY  LYS  GLN  THR  PRO  THR...
T G A A A A A A G T A A A G G C A A G C A A A C T C C G A C ...
              1930              1940              1950 ...
                                    ...  VAL  ALA  ASP  ASN  THR  ALA  ALA  THR  VAL  GLY
                                    ...C G T T G C T G A C A A T A C C G C T G C A A C C G T G G G
                                    ...              1960              1970              1980

  ASP  LEU  ARG  GLY  LEU  GLY  TRP  VAL  ILE  SER...
C G A T T T G C G C G G C T T G G G C T G G G T C A T T T C ...
              1990              2000              2010 ...
                                    ...  ALA  ASP  LYS  THR  LYS  GLY  GLU  LEU  ASN  LYS
                                    ...T G C A G A C A A A A C C A A A G G C G A A C T C A A T A A
                                    ...              2020              2030              2040

  GLU  TYR  ASN  ALA  GLN  VAL  ARG  ASN  ALA  ASN...
G G A A T A C A A C G C A C A A G T G C G T A A C G C T A A ...
              2050              2060              2070 ...
                                    ...  GLU  VAL  LYS  PHE  LYS  SER  GLY  ASN  GLY  ILE
                                    ...T G A A G T G A A A T T C A A G A G C G G C A A C G G T A T
                                    ...              2080              2090              2100
```

FIG.21K

```
   ASN  VAL  SER  GLY  LYS  THR  LEU  ASP  ASN  GLY...
C A A T G T T T C C G G T A A A A C A T T G G A T A A C G G ...
            2110              2120              2130 ...
                              ...  THR  ARG  GLU  ILE  THR  PHE  GLU  LEU  ALA  LYS
                              ...T A C G C G C G A A A T T A C T T T T G A A T T G G C T A A
                              ...            2140              2150              2160

   ASP  GLU  ASN  ALA  ILE  ALA  PHE  GLY  SER  GLY...
A G A C G A A A A T G C C A T T G C T T T C G G T T C T G G ...
            2170              2180              2190 ...
                              ...  SER  LYS  ALA  LEU  ARG  ASP  ASN  THR  VAL  ALA
                              ...C T C A A A A G C C T T G C G C G A T A A C A C G G T G G C
                              ...            2200              2210              2220

   ILE  GLY  THR  GLY  ASN  VAL  VAL  ASN  ALA  GLU...
A A T T G G T A C G G G C A A C G T T G T G A A T G C G G A ...
            2230              2240              2250 ...
                              ...  LYS  SER  GLY  ALA  PHE  GLY  ASP  PRO  ASN  TYR
                              ...A A A A T C T G G T G C A T T C G G C G A T C C G A A C T A
                              ...            2260              2270              2280

   ILE  GLU  ASP  LYS  ALA  GLY  GLY  SER  TYR  ALA...
C A T C G A A G A T A A A G C C G G T G G C A G C T A C G C ...
            2290              2300              2310 ...
```

FIG.21L

```
                                    ...  PHE  GLY  ASN  ASP  ASN  ARG  ILE  THR  SER  LYS
                                    ...T T T C G G T A A C G A T A A C C G T A T T A C T T C T A A
                                    ...            2320            2330            2340

  ASN  THR  PHE  VAL  LEU  GLY  ASN  SER  VAL  ASN...
A A A C A C T T T T G T G T T G G G T A A T A G T G T T A A ...
            2350            2360            2370 ...
                                    ...  ALA  LYS  ARG  ASP  ALA  ASN  GLY  ASN  VAL  LEU
                                    ...T G C G A A A C G T G A T G C A A A T G G C A A T G T A C T
                                    ...            2380            2390            2400

  THR  GLU  GLU  LYS  GLU  VAL  VAL  GLY  LYS  ASP...
G A C C G A A G A A A A A G A A G T G G T T G G A A A A G A ...
            2410            2420            2430 ...
                                    ...  GLY  ALA  LYS  THR  LYS  VAL  THR  VAL  PRO  GLN
                                    ...C G G T G C G A A G A C G A A A G T A A C C G T G C C G C A
                                    ...            2440            2450            2460

  ALA  LEU  GLY  GLU  THR  VAL  GLU  ASN  SER  VAL...
A G C C T T A G G C G A A A C C G T A G A A A A T T C T G T ...
            2470            2480            2490 ...
                                    ...  TYR  LEU  GLY  ASN  ALA  SER  THR  ALA  THR  LYS
                                    ...T T A T C T C G G T A A T G C T T C A A C T G C G A C A A A
                                    ...            2500            2510            2520
```

FIG.21M

```
   ASP  LYS  GLY  LYS  ASN  LEU  LYS  SER  ASP  GLY...
A G A T A A G G G T A A A A A C C T G A A A T C T G A T G G ...
            2530              2540              2550 ...
                              ...  THR  ALA  GLY  ASN  THR  THR  THR  ALA  GLY  ALA
                              ...T A C G G C G G G T A A C A C T A C A A C T G C T G G C G C
                              ...            2560              2570              2580

   THR  GLY  THR  VAL  ASN  GLY  PHE  ALA  GLY  ALA...
A A C G G G T A C G G T A A A C G G C T T T G C C G G T G C ...
            2590              2600              2610 ...
                              ...  THR  ALA  HIS  GLY  ALA  VAL  SER  VAL  GLY  ALA
                              ...A A C G G C G C A C G G T G C G G T T T C T G T C G G C G C
                              ...            2620              2630              2640

   SER  GLY  GLU  GLU  ARG  ARG  ILE  GLN  ASN  VAL...
A A G T G G C G A A G A A A G A C G T A T C C A A A A C G T ...
            2650              2660              2670 ...
                              ...  ALA  ALA  GLY  GLU  ILE  SER  ALA  THR  SER  THR
                              ...C G C G G C A G G C G A A A T T T C C G C T A C T T C C A C
                              ...            2680              2690              2700

   ASP  ALA  ILE  ASN  GLY  SER  GLN  LEU  TYR  ALA...
A G A T G C G A T T A A C G G T A G C C A G T T G T A T G C ...
            2710              2720              2730 ...
```

FIG.21N

```
                                    ...  VAL  ALA  LYS  GLY  VAL  THR  ASN  LEU  ALA  GLY
                                    ...T G T G G C A A A A G G G G T A A C A A A C C T T G C T G G
                                    ...              2740              2750              2760

  GLN  VAL  ASN  LYS  VAL  GLY  LYS  ARG  ALA  ASP...
A C A A G T G A A T A A A G T G G G C A A A C G T G C A G A ...
            2770              2780              2790 ...
                                    ...  ALA  GLY  THR  ALA  SER  ALA  LEU  ALA  ALA  SER
                                    ...T G C A G G T A C A G C A A G T G C A T T A G C G G C T T C
                                    ...              2800              2810              2820

  GLN  LEU  PRO  GLN  ALA  SER  MET  PRO  GLY  LYS...
A C A G T T A C C A C A A G C C T C T A T G C C A G G T A A ...
            2830              2840              2850 ...
                                    ...  SER  MET  VAL  SER  ILE  ALA  GLY  SER  SER  TYR
                                    ...A T C A A T G G T T T C T A T T G C G G G A A G T A G T T A
                                    ...              2860              2870              2880

  GLN  GLY  GLN  SER  GLY  LEU  ALA  ILE  GLY  VAL...
T C A A G G T C A A A G T G G T T T A G C T A T C G G G G T ...
            2890              2900              2910 ...
                                    ...  SER  ARG  ILE  SER  ASP  ASN  GLY  LYS  VAL  ILE
                                    ...A T C A A G A A T T T C C G A T A A T G G C A A A G T G A T
                                    ...              2920              2930              2940
```

FIG.21O

```
   ILE   ARG   LEU   SER   GLY   THR   THR   ASN   SER   GLN...
 T A T T C G C T T G T C A G G C A C A A C C A A T A G C C A ...
               2950                2960                2970 ...
                                    ...  GLY   LYS   THR   GLY   VAL   ALA   ALA   GLY   VAL   GLY
                                    ...A G G T A A A A C A G G C G T T G C A G C A G G T G T T G G
                                    ...             2980                2990                3000

   TYR   GLN   TRP   ***   ***   ASN   SER   GLY   SER
 T T A C C A G T G G T A A T A G A A T T C C G G A T C C G C
               3010                3020                3030
```

FIG.22A

NTHi strain K9 hia sequence

```
 MET  ASN  LYS  ILE  PHE  ASN  VAL  ILE  TRP  ASN ...
A T G A A C A A A A T T T T T A A C G T T A T T T G G A A T...
              10                   20                   30...
                                ... VAL  MET  THR  GLN  THR  TRP  ALA  VAL  VAL  SER
                                ... G T T A T G A C T C A A A C T T G G G C T G T C G T A T C T
                                ...                  40                   50                   60

 GLU  LEU  THR  ARG  ALA  HIS  THR  LYS  ARG  ALA ...
G A A C T C A C T C G C G C C C A C A C C A A A C G T G C C...
              70                   80                   90...
                                ... SER  ALA  THR  VAL  ALA  THR  ALA  VAL  LEU  ALA
                                ... T C C G C A A C C G T G G C G A C C G C C G T A T T G G C G
                                ...                  100                  110                  120

 THR  GLN  LEU  SER  ALA  THR  ALA  GLU  ALA  ASN ...
A C G C A G T T G T C T G C A A C G G C T G A A G C G A A C...
              130                  140                  150...
                                ... SER  SER  ALA  SER  VAL  THR  SER  ARG  LEU  ASN
                                ... A G T A G T G C T T C T G T T A C G A G T A G G T T G A A T
                                ...                  160                  170                  180

 VAL  TYR  GLY  ASP  THR  ASN  THR  LYS  PHE  ASN ...
G T T T A T G G C G A T A C G A A T A C T A A A T T C A A T...
              190                  200                  210...
```

FIG.22B

```
                                    ... ALA  ALA  ASN  ASN  SER  ILE  ALA  ASP  LEU  ASN
                                    ... G C A G C C A A T A A T T C A A T A G C A G A T T T A A A T
                                    ...              220                  230                  240

 LYS  GLN  ASN  ASP  GLY  VAL  HIS  ASP  GLY  LEU ...
A A A C A A A A T G A T G G T G T T C A C G A T G G T T T A...
               250                  260                  270...
                                    ... LEU  ASN  LEU  ASN  GLU  ASN  GLY  ALA  ASN  LYS
                                    ... T T A A A T C T G A A T G A A A A C G G T G C G A A T A A A
                                    ...              280                  290                  300

 LYS  LEU  LEU  VAL  ASP  ASP  ASN  THR  ALA  ALA ...
A A G C T G T T G G T G G A T G A C A A T A C T G C G G C G...
               310                  320                  330...
                                    ... THR  VAL  GLY  ASP  LEU  ARG  LYS  LEU  GLY  TRP
                                    ... A C C G T A G G C G A T T T A C G T A A A T T G G G C T G G
                                    ...              340                  350                  360

 VAL  VAL  SER  THR  LYS  ASN  GLY  LYS  GLU  ASN ...
G T C G T A T C A A C C A A A A A T G G C A A G G A A A A T...
               370                  380                  390...
                                    ... GLU  LYS  SER  GLN  GLN  VAL  LYS  GLN  ALA  ASP
                                    ... G A G A A A A G C C A A C A A G T C A A A C A G G C G G A T
                                    ...              400                  410                  420
```

FIG.22C

```
 GLU  VAL  LEU  PHE  LYS  GLY  SER  LYS  GLY  GLY ...
G A A G T G T T G T T T A A A G G C A G C A A A G G C G G T...
             430              440              450...
                                ... VAL  GLN  VAL  THR  SER  THR  SER  GLU  ASN  GLY
                                ... G T G C A G G T T A C T T C C A C C T C T G A A A A C G G C
                                ...              460              470              480

 LYS  HIS  ALA  ILE  THR  PHE  ALA  LEU  ALA  LYS ...
A A A C A C G C C A T T A C C T T T G C T T T A G C G A A A...
             490              500              510...
                                ... ASP  LEU  ASP  MET  ARG  THR  ALA  THR  VAL  SER
                                ... G A C C T T G A T A T G A G A A C T G C G A C T G T G A G T
                                ...              520              530              540

 ASP  THR  LEU  THR  ILE  GLY  GLY  SER  THR  THR ...
G A T A C C T T A A C G A T T G G C G G T A G T A C T A C T...
             550              560              570...
                                ... THR  GLY  SER  ALA  THR  THR  PRO  LYS  VAL  ASN
                                ... A C A G G T A G T G C A A C A A C A C C A A A A G T G A A T
                                ...              580              590              600

 VAL  THR  SER  THR  ALA  SER  GLY  LEU  ASN  PHE ...
G T G A C T A G C A C G G C A A G C G G C T T G A A C T T T...
             610              620              630...
```

FIG.22D

```
... ALA LYS GLY ALA THR GLY ALA ASN GLY ASP
... G C G A A A G G C G C T A C A G G T G C T A A T G G C G A T
...                640                650                660

 THR THR VAL HIS LEU THR ASN ILE ALA SER ...
A C T A C G G T T C A C T T G A C T A A T A T T G C T T C A...
          670                680                690...
                ... THR LEU GLN ASP THR LEU LEU ASN THR GLY
                ... A C T T T G C A A G A T A C T C T A T T G A A T A C T G G G
                ...                700                710                720

 VAL VAL SER LYS LEU ASP GLY ASN GLY ILE ...
G T T G T G A G T A A A T T A G A T G G T A A T G G T A T T...
          730                740                750...
                ... THR ALA ASP GLU LYS LYS ARG ALA ALA SER
                ... A C T G C T G A C G A G A A A A A A C G T G C G G C A A G C
                ...                760                770                780

 VAL GLN ASP VAL LEU ASN SER GLY TRP ASN ...
G T T C A A G A T G T T T T A A A T A G T G G T T G G A A T...
          790                800                810...
                ... ILE LYS GLY VAL LYS THR GLY ALA THR THR
                ... A T C A A G G G T G T T A A A A C A G G T G C G A C G A C T
                ...                820                830                840
```

FIG.22E

```
 SER  ASP  ASN  VAL  ASP  PHE  VAL  ARG  THR  TYR ...
T C T G A T A A C G T T G A T T T T G T C C G T A C T T A C...
              850                  860                  870...
                                ... ASP  THR  VAL  GLU  PHE  LEU  SER  GLY  SER  GLU
                                ... G A C A C A G T T G A G T T T T T G A G C G G A A G T G A A
                                ...                  880                  890                  900

 GLU  THR  THR  LEU  VAL  THR  VAL  ASP  SER  GLU ...
G A A A C T A C A C T G G T T A C A G T G G A T A G T G A A...
              910                  920                  930...
                                ... SER  ASN  GLY  LYS  SER  THR  LYS  VAL  LYS  ILE
                                ... A G T A A T G G A A A A T C T A C T A A A G T T A A A A T C
                                ...                  940                  950                  960

 GLY  ALA  LYS  THR  SER  VAL  ILE  LYS  GLU  LYS ...
G G T G C G A A G A C C T C T G T T A T C A A A G A A A A A...
              970                  980                  990...
                                ... ASP  GLY  LYS  LEU  PHE  THR  GLY  LYS  ALA  ASN
                                ... G A C G G T A A G T T A T T T A C T G G A A A A G C T A A T
                                ...                 1000                 1010                 1020

 LYS  ASP  THR  ASN  GLN  VAL  ALA  SER  ASN  ASN ...
A A A G A C A C A A A T C A A G T C G C A A G T A A T A A T...
              1030                 1040                 1050...
```

FIG.22F

```
                                  ... ALA  ALA  ASP  ASP  THR  ASP  GLU  GLY  LYS  GLY
                                  ... G C A G C T G A T G A T A C G G A T G A G G G C A A A G G C
                                  ...              1060              1070              1080

 LEU  VAL  THR  ALA  GLU  THR  VAL  ILE  ASN  ALA ...
T T A G T C A C T G C A G A G A C T G T T A T C A A T G C A...
            1090              1100              1110...
                                  ... VAL  ASN  LYS  ALA  GLY  TRP  ARG  ILE  LYS  THR
                                  ... G T A A A C A A G G C T G G T T G G A G A A T T A A A A C A
                                  ...              1120              1130              1140

 THR  GLY  ALA  ASN  ASN  GLN  ALA  GLY  GLN  PHE ...
A C G G G T G C T A A T A A T C A A G C T G G T C A G T T T...
            1150              1160              1170...
                                  ... GLU  THR  VAL  THR  SER  GLY  THR  ASN  VAL  THR
                                  ... G A A A C T G T C A C A T C A G G C A C A A A T G T A A C C
                                  ...              1180              1190              1200

 PHE  ALA  ASP  GLY  ASN  GLY  THR  THR  ALA  VAL ...
T T T G C T G A T G G C A A T G G T A C A A C T G C A G T C...
            1210              1220              1230...
                                  ... VAL  THR  GLY  ASP  ALA  THR  ASN  GLY  ILE  THR
                                  ... G T A A C T G G C G A T G C T A C C A A T G G T A T T A C T
                                  ...              1240              1250              1260
```

FIG.22G

```
 VAL   LYS   TYR   GLU   ALA   LYS   VAL   GLY   ASP   GLY ...
G T T A A A T A C G A A G C G A A A G T T G G C G A C G G C...
             1270                1280                1290...
                                ... LEU   LYS   ILE   GLY   ASN   ASP   GLN   LYS   ILE   THR
                                ... T T G A A G A T T G G T A A C G A C C A A A A A A T C A C T
                                ...                 1300                1310                1320

 ALA   ASP   THR   THR   ALA   LEU   THR   VAL   THR   GLY ...
G C A G A T A C G A C C G C A C T T A C T G T G A C G G G C...
             1330                1340                1350...
                                ... GLY   LYS   VAL   THR   ALA   PRO   ASP   ALA   THR   ASN
                                ... G G T A A A G T T A C T G C C C C T G A T G C A A C C A A T
                                ...                 1360                1370                1380

 GLY   LYS   LYS   LEU   VAL   ASN   ALA   SER   GLY   LEU ...
G G T A A G A A A C T T G T T A A T G C A A G T G G T T T A...
             1390                1400                1410...
                                ... ALA   ASP   ALA   LEU   ASN   LYS   LEU   SER   TRP   THR
                                ... G C T G A T G C G T T A A A C A A A T T A A G T T G G A C T
                                ...                 1420                1430                1440

 ALA   LYS   ALA   GLU   ALA   ASP   THR   ALA   ASN   GLY ...
G C A A A A G C T G A A G C A G A T A C T G C T A A T G G C...
             1450                1460                1470...
```

FIG.22H

```
                                    ... GLY  GLU  LEU  ASP  GLY  THR  ALA  ASP  GLU  LYS
                                    ... G G C G A G C T T G A T G G A A C T G C A G A T G A A A A A
                                    ...              1480              1490              1500

 GLU  VAL  LYS  ALA  GLY  GLU  THR  VAL  THR  PHE ...
G A A G T T A A A G C A G G C G A A A C G G T A A C C T T T...
            1510              1520              1530...
                                    ... LYS  ALA  GLY  LYS  ASN  LEU  LYS  VAL  LYS  GLN
                                    ... A A A G C G G G C A A G A A C T T A A A A G T G A A A C A A
                                    ...              1540              1550              1560

 ASP  GLY  ALA  ASN  PHE  THR  TYR  SER  LEU  GLN ...
G A T G G T G C G A A C T T T A C T T A T T C A C T G C A A...
            1570              1580              1590...
                                    ... ASP  ALA  LEU  THR  GLY  LEU  THR  SER  ILE  THR
                                    ... G A T G C T T T A A C A G G C T T A A C G A G C A T T A C T
                                    ...              1600              1610              1620

 LEU  GLY  THR  GLY  ASN  ASN  GLY  ALA  LYS  THR ...
T T A G G T A C A G G A A A T A A T G G T G C G A A A A C T...
            1630              1640              1650...
                                    ... GLU  ILE  ASN  LYS  ASP  GLY  LEU  THR  ILE  THR
                                    ... G A A A T C A A C A A A G A C G G C T T A A C C A T C A C A
                                    ...              1660              1670              1680
```

FIG.22I

```
 PRO  ALA  ASN  GLY  ALA  GLY  ALA  ASN  ASN  ALA ...
C C A G C A A A T G G T G C G G G T G C A A A T A A T G C A...
             1690              1700              1710...
                               ... ASN  THR  ILE  SER  VAL  THR  LYS  ASP  GLY  ILE
                               ... A A C A C C A T C A G C G T A A C C A A A G A C G G C A T T
                               ...              1720              1730              1740

 SER  ALA  GLY  GLY  GLN  SER  VAL  LYS  ASN  VAL ...
A G T G C G G G C G G T C A G T C G G T T A A A A A C G T T...
             1750              1760              1770...
                               ... VAL  SER  GLY  LEU  LYS  LYS  PHE  GLY  ASP  ALA
                               ... G T G A G C G G A C T G A A G A A A T T T G G T G A T G C G
                               ...              1780              1790              1800

 ASN  PHE  ASP  PRO  LEU  THR  SER  SER  ALA  ASP ...
A A T T T C G A T C C G C T G A C T A G C T C C G C C G A C...
             1810              1820              1830...
                               ... ASN  LEU  THR  LYS  GLN  TYR  ASP  ASP  ALA  TYR
                               ... A A C T T A A C G A A A C A A T A T G A C G A T G C C T A T
                               ...              1840              1850              1860

 LYS  GLY  LEU  THR  ASN  LEU  ASP  GLU  LYS  GLY ...
A A A G G C T T G A C C A A T T T G G A T G A A A A A G G T...
             1870              1880              1890...
```

FIG.22J

```
                                    ... ALA  ASP  LYS  GLN  THR  LEU  THR  VAL  ALA  ASP
                                    ... G C G G A C A A G C A A A C T C T G A C T G T T G C C G A C
                                    ...              1900              1910              1920

 ASN  THR  ALA  ALA  THR  VAL  GLY  ASP  LEU  ARG ...
A A T A C T G C C G C A A C C G T G G G C G A T T T G C G C...
            1930              1940              1950...
                                    ... GLY  LEU  GLY  TRP  VAL  ILE  SER  ALA  ASP  LYS
                                    ... G G C T T G G G C T G G G T C A T T T C T G C G G A C A A A
                                    ...              1960              1970              1980

 THR  THR  GLY  GLU  LEU  ASP  LYS  GLU  TYR  ASN ...
A C C A C A G G C G A A C T C G A T A A G G A A T A C A A C...
            1990              2000              2010...
                                    ... ALA  GLN  VAL  ARG  ASN  ALA  ASN  GLU  VAL  LYS
                                    ... G C G C A A G T G C G T A A C G C C A A T G A A G T G A A A
                                    ...              2020              2030              2040

 PHE  LYS  SER  GLY  ASN  GLY  ILE  ASN  VAL  SER ...
T T C A A A A G C G G C A A C G G T A T C A A T G T T T C C...
            2050              2060              2070...
                                    ... GLY  LYS  THR  VAL  ASN  GLY  ARG  ARG  GLU  ILE
                                    ... G G T A A A A C T G T C A A C G G T A G G C G T G A A A T T
                                    ...              2080              2090              2100
```

FIG.22K

```
 THR  PHE  GLU  LEU  ALA  LYS  GLY  GLU  VAL  VAL ...
A C T T T T G A A T T G G C T A A A G G C G A A G T G G T T...
            2110              2120              2130...
                              ... LYS  SER  ASN  GLU  PHE  THR  VAL  LYS  GLU  THR
                              ... A A A T C G A A T G A A T T T A C T G T C A A A G A A A C C
                              ...            2140              2150              2160

 ASN  GLY  LYS  GLU  THR  SER  LEU  VAL  LYS  VAL ...
A A T G G C A A G G A A A C G A G C C T G G T T A A A G T T...
            2170              2180              2190...
                              ... GLY  ASP  LYS  TYR  TYR  SER  LYS  GLU  ASP  ILE
                              ... G G C G A T A A A T A T T A C A G C A A A G A G G A T A T T
                              ...            2200              2210              2220

 ASP  PRO  ALA  THR  GLY  LYS  PRO  LYS  VAL  THR ...
G A C C C A G C A A C C G G T A A A C C G A A A G T T A C A...
            2230              2240              2250...
                              ... ASN  GLY  ASN  ALA  VAL  ALA  ALA  LYS  TYR  GLN
                              ... A A T G G C A A T G C A G T T G C T G C G A A A T A T C A A
                              ...            2260              2270              2280

 ASP  LYS  ASP  GLY  LYS  VAL  VAL  SER  ALA  ASP ...
G A T A A A G A T G G C A A A G T C G T T T C T G C T G A C...
            2290              2300              2310...
```

FIG.22L

```
                                    ... GLY  SER  SER  ASN  THR  ALA  VAL  THR  LEU  THR
                                    ... G G C A G C A G C A A T A C C G C T G T T A C C C T A A C C
                                    ...              2320              2330              2340

 ASN  LYS  GLY  TYR  GLY  TYR  VAL  THR  GLY  ASN ...
A A C A A A G G T T A T G G C T A T G T A A C A G G T A A C...
           2350              2360              2370...
                                    ... GLN  VAL  ALA  ASP  ALA  ILE  ALA  LYS  SER  GLY
                                    ... C A A G T G G C A G A T G C G A T T G C G A A A T C A G G C
                                    ...              2380              2390              2400

 PHE  GLU  LEU  GLY  LEU  ALA  ASP  ALA  GLU  LYS ...
T T T G A G C T T G G T T T G G C T G A T G C A G A A A A A...
           2410              2420              2430...
                                    ... ALA  LYS  ALA  ALA  PHE  GLY  ASP  GLU  THR  LYS
                                    ... G C G A A A G C T G C G T T T G G C G A T G A A A C A A A A
                                    ...              2440              2450              2460

 ALA  LEU  SER  SER  ASP  LYS  LEU  GLU  THR  VAL ...
G C C T T G T C T T C T G A T A A A T T G G A A A C C G T A...
           2470              2480              2490...
                                    ... ASN  ALA  ASN  ASP  LYS  VAL  ARG  PHE  ALA  ASN
                                    ... A A T G C C A A C G A C A A A G T C C G T T T T G C T A A T
                                    ...              2500              2510              2520
```

FIG.22M

```
 GLY  LEU  ASN  THR  LYS  VAL  SER  ALA  ALA  THR ...
G G T T T A A A T A C C A A A G T G A G C G C G G C A A C G...
            2530              2540              2550...
                              ... VAL  GLU  SER  ILE  ASP  ALA  ASN  GLY  ASP  LYS
                              ... G T G G A A A G C A T C G A T G C A A A C G G C G A T A A A
                              ...            2560              2570              2580

 VAL  THR  THR  THR  PHE  VAL  LYS  THR  ASP  VAL ...
G T G A C T A C A A C C T T T G T G A A A A C C G A T G T G...
            2590              2600              2610...
                              ... GLU  LEU  PRO  LEU  THR  GLN  ILE  TYR  ASN  THR
                              ... G A A T T G C C T T T A A C G C A A A T C T A C A A T A C C
                              ...            2620              2630              2640

 ASP  ALA  ASN  GLY  LYS  LYS  ILE  VAL  LYS  ASN ...
G A T G C A A A C G G T A A G A A A A T C G T T A A A A A T...
            2650              2660              2670...
                              ... GLY  ASP  LYS  TRP  TYR  TYR  THR  LYS  ASP  ASP
                              ... G G C G A T A A A T G G T A T T A C A C G A A A G A T G A C
                              ...            2680              2690              2700

 GLY  SER  THR  ASP  MET  THR  LYS  GLU  VAL  THR ...
G G C T C A A C T G A T A T G A C T A A A G A A G T T A C C...
            2710              2720              2730...
```

FIG.22N

```
                                    ... LEU GLY ASN VAL ASP SER ASP GLY LYS LYS
                                    ... C T T G G C A A T G T G G A T T C A G A C G G C A A G A A A
                                    ...              2740             2750             2760

 VAL VAL LYS GLU ASP ASN LYS TRP TYR HIS ...
G T T G T G A A A G A A G A C A A C A A G T G G T A T C A C...
             2770             2780             2790...
                                    ... VAL LYS SER ASP GLY SER THR ASP LYS THR
                                    ... G T T A A A T C T G A T G G T T C T A C G G A T A A A A C A
                                    ...              2800             2810             2820

 GLN VAL VAL GLU GLU ALA LYS VAL SER THR ...
C A G G T G G T C G A A G A A G C T A A A G T T T C T A C C...
             2830             2840             2850...
                                    ... ASP GLU LYS HIS VAL VAL SER LEU ASP PRO
                                    ... G A T G A A A A A C A C G T T G T C A G C C T T G A T C C A
                                    ...              2860             2870             2880

 ASN ASP GLN SER LYS GLY LYS GLY VAL VAL ...
A A T G A T C A A T C A A A A G G T A A A G G C G T G G T C...
             2890             2900             2910...
                                    ... ILE ASN ASN MET ALA ASN GLY GLU ILE SER
                                    ... A T T A A C A A T A T G G C T A A T G G C G A A A T T T C T
                                    ...              2920             2930             2940
```

FIG.22O

```
 ALA  THR  SER  THR  ASP  ALA  ILE  ASN  GLY  SER ...
G C C A C T T C C A C C G A T G C G A T T A A C G G A A G T...
          2950           2960           2970...
                         ... GLN  LEU  TYR  ALA  VAL  ALA  LYS  GLY  VAL  THR
                         ... C A G T T G T A T G C C G T G G C A A A A G G G G T A A C A
                         ...          2980           2990           3000

 ASN  LEU  ALA  GLY  GLN  VAL  ASN  ASN  LEU  GLU ...
A A C C T T G C T G G A C A A G T G A A T A A T C T T G A G...
          3010           3020           3030...
                         ... GLY  LYS  VAL  ASN  LYS  VAL  GLY  LYS  ARG  ALA
                         ... G G C A A A G T G A A T A A A G T G G G C A A A C G T G C A
                         ...          3040           3050           3060

 ASP  ALA  GLY  THR  ALA  SER  ALA  LEU  ALA  ALA ...
G A T G C A G G T A C T G C A A G T G C A T T A G C G G C T...
          3070           3080           3090...
                         ... SER  GLN  LEU  PRO  GLN  ALA  THR  MET  PRO  GLY
                         ... T C A C A G T T A C C A C A A G C C A C T A T G C C A G G T
                         ...          3100           3110           3120

 LYS  SER  MET  VAL  SER  ILE  ALA  GLY  SER  SER ...
A A A T C A A T G G T T T C T A T T G C G G G A A G T A G T...
          3130           3140           3150...
```

FIG.22P

```
                                    ... TYR  GLN  GLY  GLN  ASN  GLY  LEU  ALA  ILE  GLY
                                    ... T A T C A A G G T C A A A A T G G T T T A G C T A T C G G G
                                    ...              3160              3170              3180

 VAL  SER  ARG  ILE  SER  ASP  ASN  GLY  LYS  VAL ...
G T A T C A A G A A T T T C C G A T A A T G G C A A A G T G...
             3190              3200              3210...
                                    ... ILE  ILE  ARG  LEU  SER  GLY  THR  THR  ASN  SER
                                    ... A T T A T T C G C T T G T C A G G C A C A A C C A A T A G T
                                    ...              3220              3230              3240

 GLN  GLY  LYS  THR  GLY  VAL  ALA  ALA  GLY  VAL ...
C A A G G T A A A A C A G G C G T T G C A G C A G G T G T T...
             3250              3260              3270...
                                    ... GLY  TYR  GLN  TRP  ***
                                    ... G G T T A C C A G T G G T A A T A G A A T T C C G G A T C C
                                    ...              3280              3290              3300
```

FIG.23A

NTHi strain K22 Hia

```
                    MET  ASN  LYS  ILE  PHE  ASN...
G C G A A T T C A T A T G A A C A A A A T T T T T A A ...
              10                  20                ...
                                ...  VAL  ILE  TRP  ASN  VAL  VAL  THR  GLN  THR  TRP  VAL
                                ...C G T T A T T T G G A A T G T T G T G A C T C A A A C T T G G G T
                                ...  30                  40                  50                  60

   VAL  VAL  SER  GLU  LEU  THR  ARG  ALA  HIS...
T G T C G T A T C T G A A C T C A C T C G C G C C C A ...
              70                  80                ...
                                ...  THR  LYS  CYS  ALA  SER  ALA  THR  VAL  ALA  VAL  ALA
                                ...C A C C A A A T G C G C C T C C G C C A C C G T G G C G G T T G C
                                ...  90                  100                 110                 120

   VAL  LEU  ALA  THR  ALA  LEU  SER  ALA  THR...
C G T A T T G G C A A C T G C G T T G T C T G C A A C ...
             130                 140                ...
                                ...  ALA  GLU  ALA  ASN  ASN  ASN  THR  SER  VAL  THR  ASN
                                ...G G C T G A A G C G A A C A A C A A T A C T T C T G T T A C G A A
                                ...  150                 160                 170                 180
```

FIG.23B

```
   GLY   LEU   ASN   ALA   TYR   GLY   ASP   THR   ASN...
T G G G T T G A A T G C T T A T G G C G A T A C T A A ...
              190                 200                 ...
                                ...   PHE   ASN   THR   THR   ASN   ASN   SER   ILE   ALA   ASP   LEU
                                ...T T T T A A T A C A A C C A A T A A T T C G A T A G C A G A T T T
                                ...  210                 220                 230                 240

   GLU   LYS   HIS   VAL   GLN   ASP   ALA   TYR   LYS...
G G A A A A A C A C G T T C A A G A T G C T T A T A A ...
              250                 260                 ...
                                ...   GLY   LEU   LEU   ASN   LEU   ASN   GLU   LYS   ASP   THR   ASN
                                ...A G G C T T A T T A A A T C T G A A T G A A A A A G A T A C A A A
                                ...  270                 280                 290                 300

   LYS   SER   SER   PHE   LEU   VAL   ALA   ASP   ASN...
T A A G T C A A G T T T C T T G G T T G C C G A C A A ...
              310                 320                 ...
                                ...   THR   ALA   ALA   THR   VAL   GLY   ASN   LEU   ARG   LYS   LEU
                                ...T A C C G C C G C A A C C G T A G G C A A T T T G C G T A A A T T
                                ...  330                 340                 350                 360

   GLY   TRP   VAL   LEU   SER   SER   LYS   ASN   GLY...
G G G C T G G G T A T T G T C T A G C A A A A A C G G ...
              370                 380                 ...
```

FIG.23C

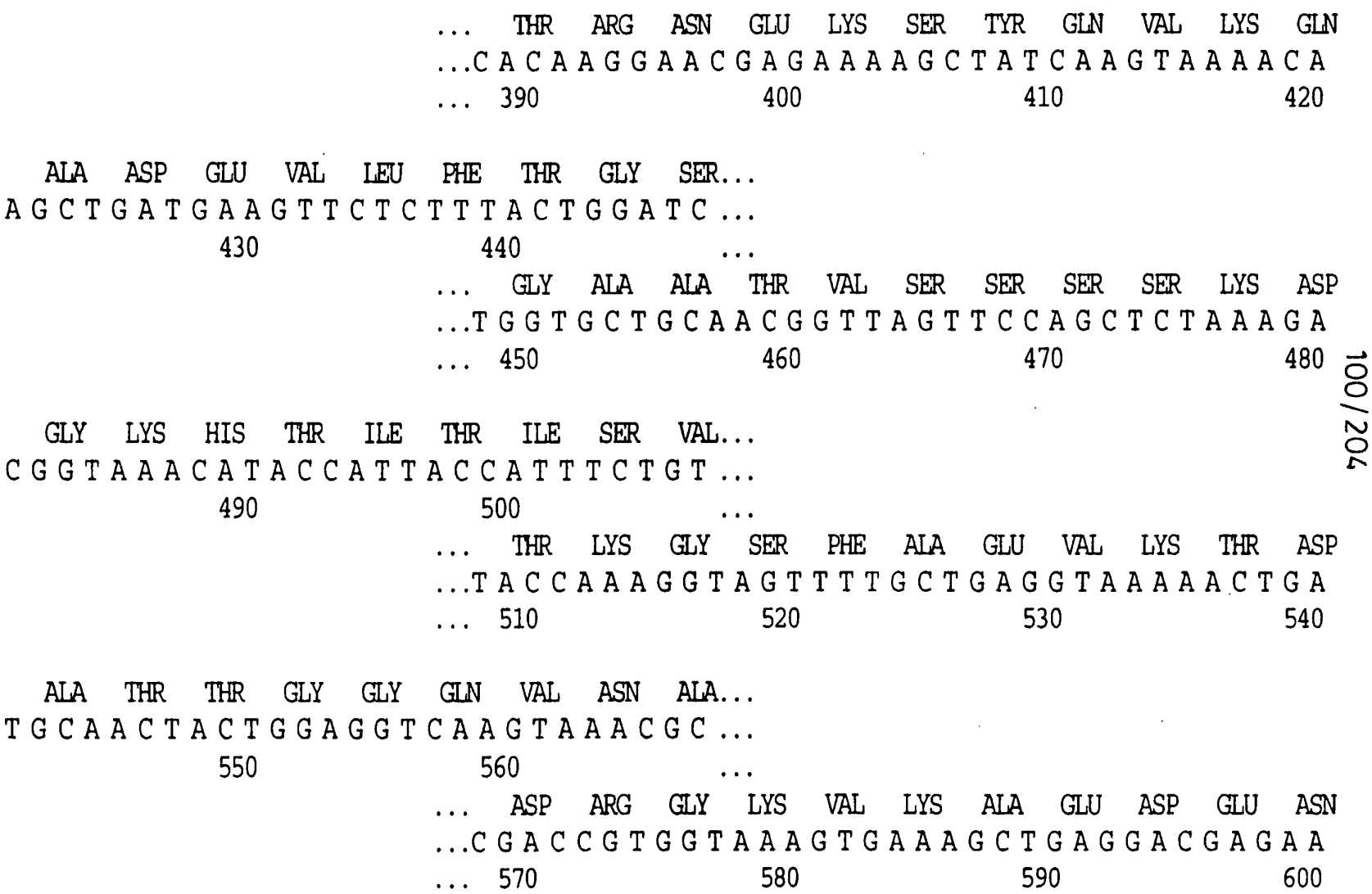


FIG.23D

```
   GLY  ALA  ASP  VAL  ASP  LYS  LYS  VAL  ALA...
TGGAGCTGATGTTGATAAGAAAGTTGC...
            610             620            ...
                                  ...  THR  VAL  LYS  ASP  VAL  ALA  LYS  ALA  ILE  ASN  ASP
                                  ...AACTGTAAAAGATGTTGCTAAGGCGATTAACGA
                                  ...  630             640             650             660

   ALA  ALA  THR  PHE  VAL  LYS  VAL  GLU  SER...
TGCCGCAACTTTCGTGAAAGTGGAAAG...
            670             680            ...
                                  ...  THR  ASP  ASP  ASP  ILE  GLU  ASN  GLY  ALA  ALA  GLY
                                  ...CACAGATGATGACATTGAAAATGGTGCTGCAGG
                                  ...  690             700             710             720

   LYS  ASN  GLU  THR  THR  ASP  GLN  ALA  LEU...
CAAAAATGAAACTACAGACCAAGCTCT...
            730             740            ...
                                  ...  LYS  ALA  GLY  ASP  THR  LEU  THR  LEU  LYS  ALA  GLY
                                  ...CAAAGCAGGCGACACCTTAACCTTAAAAGCGGG
                                  ...  750             760             770             780

   LYS  ASN  LEU  LYS  ALA  LYS  LEU  ASP  GLN...
TAAAAACTTAAAAGCTAAGTTAGACCA...
            790             800            ...
```

FIG.23E

```
                                    ...  ASN  GLY  LYS  SER  VAL  THR  PHE  ALA  LEU  ALA  LYS
                                    ...A A A T G G T A A A T C A G T A A C C T T T G C T T T A G C G A A
                                    ...  810                  820                  830                  840

  ASP  LEU  ASP  VAL  THR  SER  ALA  LYS  VAL...
A G A C C T T G A T G T G A C C T C T G C G A A A G T ...
             850                  860                  ...
                                    ...  SER  ASP  LYS  LEU  SER  ILE  GLY  LYS  ASP  THR  ASN
                                    ...G A G T G A T A A G T T G T C T A T T G G T A A A G A T A C G A A
                                    ...  870                  880                  890                  900

  LYS  VAL  ASP  ILE  THR  SER  ASP  ALA  ASN...
T A A A G T T G A T A T T A C C A G T G A T G C A A A ...
             910                  920                  ...
                                    ...  GLY  LEU  LYS  LEU  ALA  LYS  THR  GLY  ASN  GLY  ASN
                                    ...T G G C T T G A A A T T G G C G A A A A C A G G T A A C G G A A A
                                    ...  930                  940                  950                  960

  GLY  GLN  ASN  GLY  ASN  VAL  HIS  LEU  ASN...
T G G T C A A A A C G G T A A T G T C C A C T T A A A ...
             970                  980                  ...
                                    ...  GLY  ILE  ALA  SER  THR  LEU  THR  ASP  THR  ILE  THR
                                    ...T G G T A T T G C T T C G A C T T T G A C C G A T A C C A T T A C
                                    ...  990                 1000                 1010                 1020
```

FIG.23F

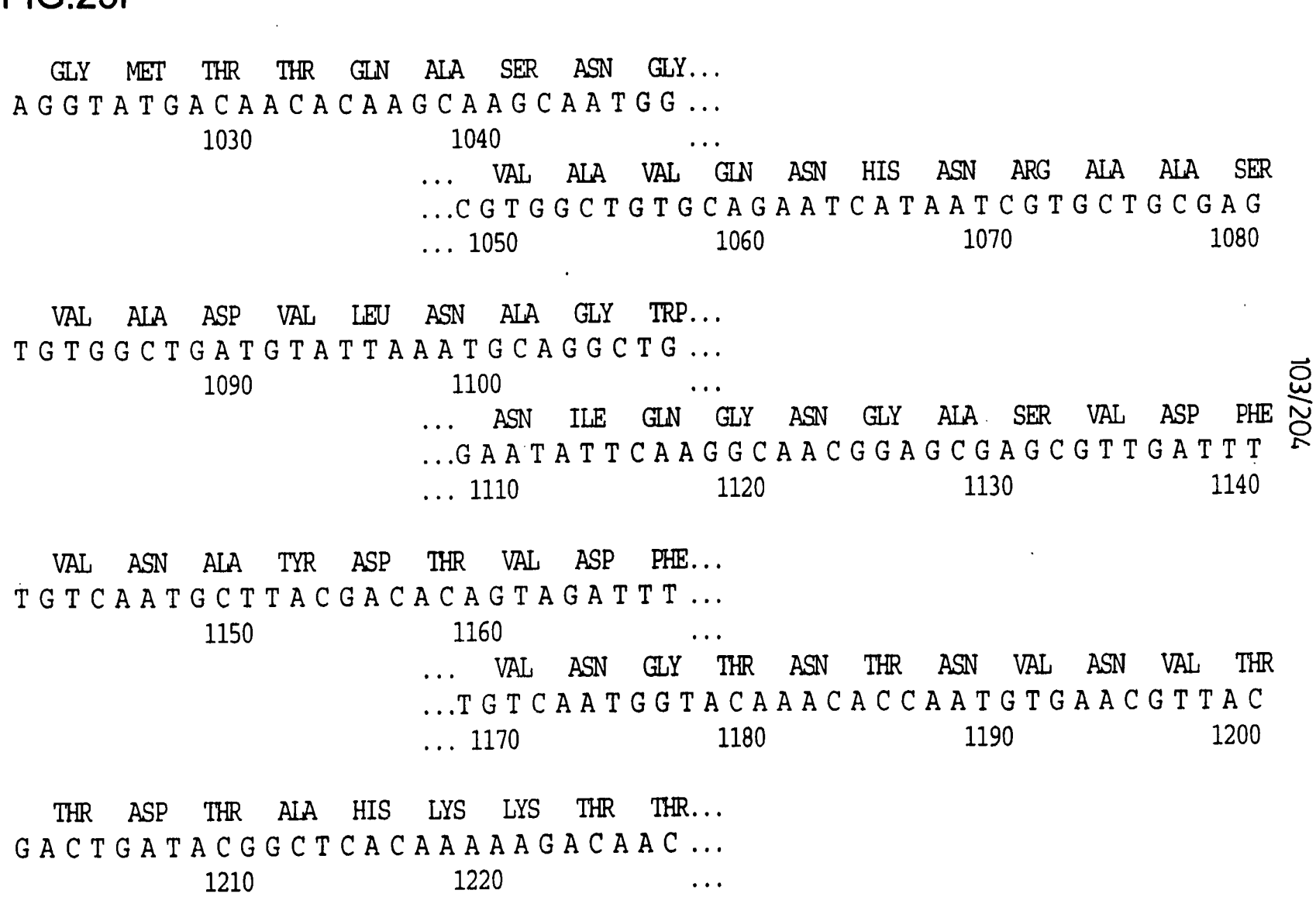


FIG.23G

```
                                  ...  VAL  ARG  VAL  ASP  VAL  THR  GLY  LEU  PRO  VAL  GLN
                                  ...C G T C C G T G T G G A T G T A A C A G G C T T G C C G G T T C A
                                  ... 1230              1240              1250              1260

  TYR  VAL  THR  GLU  ASP  GLY  LYS  THR  VAL...
A T A T G T T A C G G A A G A C G G C A A A A C C G T ...
            1270              1280              ...
                                  ...  VAL  LYS  VAL  ASP  ASN  LYS  TYR  TYR  GLU  ALA  LYS
                                  ...T G T G A A A G T G G A C A A T A A G T A T T A C G A A G C T A A
                                  ... 1290              1300              1310              1320

  GLN  ASP  GLY  SER  ALA  ASP  MET  ASP  LYS...
G C A A G A C G G T T C G G C G G A T A T G G A T A A ...
            1330              1340              ...
                                  ...  LYS  VAL  GLU  ASN  GLY  GLU  LEU  ALA  LYS  THR  LYS
                                  ...A A A A G T C G A A A A T G G C G A G C T G G C G A A A A C C A A
                                  ... 1350              1360              1370              1380

  VAL  LYS  LEU  VAL  SER  ALA  SER  GLY  GLN...
A G T G A A A T T G G T G T C G G C A A G C G G T C A ...
            1390              1400              ...
                                  ...  ASN  PRO  VAL  LYS  ILE  SER  ASN  VAL  ALA  GLU  GLY
                                  ...A A A T C C G G T G A A A A T C A G C A A T G T T G C G G A A G G
                                  ... 1410              1420              1430              1440
```

FIG.23H

```
  THR  GLU  GLU  ASN  ASP  ALA  VAL  SER  PHE...
CACGGAAGAAAACGATGCGGTCAGCTT ...
          1450           1460          ...
                                  ...  LYS  GLN  LEU  LYS  ALA  LEU  GLN  GLU  LYS  GLN  VAL
                                 ...TAAGCAATTGAAAGCCTTGCAAGAGAAACAGGT
                                 ... 1470          1480          1490          1500

  THR  LEU  THR  ALA  SER  ASN  ALA  TYR  ALA...
TACTTTAACTGCGAGCAATGCTTATGC ...
          1510           1520          ...
                                  ...  ASN  GLY  GLY  ASN  ASP  ALA  ASP  GLY  GLY  LYS  ALA
                                 ...CAATGGTGGTAACGATGCCGACGGCGGCAAGGC
                                 ... 1530          1540          1550          1560

  THR  GLN  THR  LEU  ASN  ASN  GLY  LEU  ASN...
AACTCAAACTTTAAACAATGGTTTGAA ...
          1570           1580          ...
                                  ...  PHE  LYS  PHE  LYS  SER  THR  ASP  GLY  GLU  LEU  LEU
                                 ...TTTTAAATTTAAATCCACAGACGGCGAGTTGTT
                                 ... 1590          1600          1610          1620

  ASN  ILE  LYS  VAL  GLU  ASN  ASP  THR  VAL...
GAACATCAAAGTAGAAAATGACACAGT ...
          1630           1640          ...
```

FIG.23I

```
                                ...  THR  PHE  THR  PRO  LYS  LYS  GLY  SER  VAL  GLN  VAL
                                ...T A C C T T T A C G C C G A A A A A A G G T T C G G T A C A G G T
                                ... 1650              1660              1670              1680

  GLY  GLU  ASP  GLY  LYS  ALA  THR  ILE  GLN...
T G G C G A A G A C G G T A A G G C T A C G A T T C A ...
          1690              1700              ...
                                ...  ASN  GLY  THR  LYS  THR  THR  ASP  GLY  LEU  VAL  GLU
                                ...A A A T G G T A C G A A A A C A A C C G A C G G T T T G G T T G A
                                ... 1710              1720              1730              1740

  ALA  SER  GLU  LEU  VAL  GLU  SER  LEU  ASN...
A G C T T C C G A A T T G G T T G A A A G C C T G A A ...
          1750              1760              ...
                                ...  LYS  LEU  GLY  TRP  LYS  VAL  GLY  VAL  ASP  LYS  ASP
                                ...C A A A C T G G G C T G G A A A G T G G G C G T T G A T A A A G A
                                ... 1770              1780              1790              1800

  GLY  SER  GLY  GLU  LEU  ASP  GLY  ALA  SER...
C G G C A G C G G C G A G C T T G A T G G T G C A T C ...
          1810              1820              ...
                                ...  ASN  GLU  THR  LEU  VAL  LYS  SER  GLY  ASP  LYS  VAL
                                ...C A A T G A A A C T T T A G T G A A G T C G G G C G A T A A A G T
                                ... 1830              1840              1850              1860
```

FIG.23J

```
   THR   LEU   LYS   ALA   GLY   GLU   ASN   LEU   LYS...
A A C T T T G A A A G C C G G C G A G A A T C T G A A ...
              1870                1880                ...
                                ...   VAL   LYS   GLN   ASP   GLY   THR   ASN   PHE   THR   TYR   ALA
                                ...G G T C A A A C A A G A C G G C A C A A A C T T C A C T T A C G C
                                ... 1890                1900                1910                1920

   LEU   LYS   ASP   GLU   LEU   THR   GLY   VAL   LYS...
G C T C A A A G A T G A A T T G A C G G G C G T G A A ...
              1930                1940                ...
                                ...   SER   VAL   GLU   PHE   LYS   ASP   THR   ALA   ASN   GLY   SER
                                ...G A G C G T G G A G T T T A A A G A C A C G G C G A A T G G T T C
                                ... 1950                1960                1970                1980

   ASN   GLY   ALA   SER   THR   LYS   ILE   THR   LYS...
A A A C G G T G C A A G C A C G A A G A T T A C C A A ...
              1990                2000                ...
                                ...   ASP   GLY   LEU   THR   ILE   THR   SER   ALA   ASN   GLY   ALA
                                ...A G A C G G C T T G A C C A T T A C G T C G G C A A A C G G T G C
                                ... 2010                2020                2030                2040

   ASN   GLY   ALA   ALA   ALA   THR   ASP   ALA   ASP...
G A A T G G T G C G G C G G C G A C T G A T G C G G A ...
              2050                2060                ...
```

FIG.23K

```
                                ... LYS ILE LYS VAL ALA SER ASP GLY ILE SER ALA
                                ...C A A G A T T A A A G T G G C T T C A G A C G G C A T C A G T G C
                                ... 2070          2080          2090          2100

  GLY ASN LYS ALA VAL LYS ASN VAL VAL...
G G G T A A T A A A G C G G T T A A A A A C G T T G T ...
          2110          2120          ...
                                ... SER GLY LEU LYS LYS PHE GLY ASP ALA ASN PHE
                                ...G A G C G G A C T G A A G A A A T T T G G T G A T G C G A A T T T
                                ... 2130          2140          2150          2160

  ASN PRO LEU THR SER SER ALA ASP ASN...
C A A T C C A C T G A C C A G T T C C G C C G A C A A ...
          2170          2180          ...
                                ... LEU THR LYS GLN TYR ASP ASP ALA TYR LYS GLY
                                ...C T T A A C G A A A C A A T A T G A C G A T G C C T A T A A A G G
                                ... 2190          2200          2210          2220

  LEU THR ASN LEU ASP GLU LYS GLY ALA...
C T T G A C C A A T T T G G A T G A A A A A G G T G C ...
          2230          2240          ...
                                ... ASP LYS GLN THR LEU THR VAL ALA ASP ASN THR
                                ...G G A C A A G C A A A C T C T G A C T G T T G C C G A C A A T A C
                                ... 2250          2260          2270          2280
```

FIG.23L

```
   ALA  ALA  THR  VAL  GLY  ASP  LEU  ARG  GLY...
T G C C G C A A C C G T G G G C G A T T T G C G C G G ...
            2290              2300              ...
                                ...  LEU  GLY  TRP  VAL  ILE  SER  ALA  ASP  LYS  THR  THR
                                ...C T T G G G C T G G G T C A T T T C T G C G G A C A A A A C C A C
                                ... 2310              2320              2330              2340

   GLY  GLU  LEU  ASN  LYS  GLU  TYR  ASN  ALA...
A G G C G A A C T C A A T A A G G A A T A C A A C G C ...
            2350              2360              ...
                                ...  GLN  VAL  ARG  ASN  ALA  ASN  GLU  VAL  LYS  PHE  LYS
                                ...G C A A G T G C G T A A C G C C A A T G A A G T G A A A T T C A A
                                ... 2370              2380              2390              2400

   SER  GLY  ASN  GLY  ILE  HIS  VAL  SER  GLY...
G A G C G G C A A C G G T A T C C A T G T T T C C G G ...
            2410              2420              ...
                                ...  LYS  THR  VAL  ASN  GLY  ARG  ARG  GLU  ILE  THR  PHE
                                ...T A A A A C G G T C A A C G G T A G G C G C G A A A T T A C T T T
                                ... 2430              2440              2450              2460

   GLU  LEU  ALA  LYS  ASP  GLU  ASN  ALA  ILE...
T G A A T T G G C T A A A G A C G A A A A T G C C A T ...
            2470              2480              ...
```

FIG.23M

```
                                ...  ALA  PHE  GLY  TYR  GLY  SER  LYS  ALA  LEU  ARG  ASP
                                ...T G C T T T C G G T T A T G G C T C A A A A G C C T T G C G C G A
                                ... 2490              2500              2510              2520

  ASN  THR  VAL  ALA  ILE  GLY  THR  GLY  ASN...
T A A C A C G G T G G C A A T T G G T A C G G G C A A ...
            2530              2540              ...
                                ...  VAL  VAL  ASN  ALA  GLU  LYS  SER  GLY  ALA  PHE  GLY
                                ...C G T T G T G A A T G C G G A A A A A T C T G G T G C A T T C G G
                                ... 2550              2560              2570              2580

  ASP  PRO  ASN  TYR  ILE  GLU  ASP  LYS  ALA...
C G A T C C G A A C T A C A T C G A A G A T A A A G C ...
            2590              2600              ...
                                ...  GLY  GLY  SER  TYR  ALA  PHE  GLY  ASN  ASP  ASN  ARG
                                ...C G G T G G C A G C T A C G C T T T C G G T A A C G A T A A C C G
                                ... 2610              2620              2630              2640

  ILE  THR  SER  LYS  ASN  THR  PHE  VAL  LEU...
T A T T A C T T C T A A A A A C A C T T T T G T G T T ...
            2650              2660              ...
                                ...  GLY  ASN  GLY  VAL  ASN  ALA  LYS  TYR  LYS  ALA  ASN
                                ...G G G T A A T G G A G T T A A T G C G A A A T A T A A A G C C A A
                                ... 2670              2680              2690              2700
```

FIG.23N

```
   GLY  ASP  VAL  ASP  THR  GLU  THR  VAL  THR...
T G G A G A T G T T G A T A C G G A A A C C G T A A C ...
             2710              2720              ...
                                ...  VAL  LYS  ASP  LYS  ASP  GLY  LYS  GLU  THR  THR  VAL
                                ...C G T T A A G G A C A A A G A C G G T A A A G A G A C T A C C G T
                                ... 2730              2740              2750              2760

   THR  VAL  PRO  LYS  ALA  LEU  GLY  ALA  THR...
T A C T G T T C C T A A A G C G T T A G G G G C T A C ...
             2770              2780              ...
                                ...  VAL  GLU  ASN  SER  VAL  TYR  LEU  GLY  ASN  LYS  SER
                                ...G G T T G A A A A C T C C G T T T A T T T G G G T A A T A A A T C
                                ... 2790              2800              2810              2820

   THR  ALA  THR  LYS  ASP  LYS  GLY  LYS  ASN...
G A C T G C G A C A A A A G A T A A G G G T A A A A A ...
             2830              2840              ...
                                ...  LEU  LYS  SER  ASP  GLY  THR  ALA  GLY  ASN  THR  THR
                                ...C C T G A A A T C T G A T G G T A C G G C G G G T A A C A C T A C
                                ... 2850              2860              2870              2880

   THR  ALA  GLY  THR  THR  GLY  THR  VAL  ASN...
A A C T G C T G G C A C A A C G G G T A C G G T A A A ...
             2890              2900              ...
```

FIG.23O

```
                                ...  GLY  PHE  ALA  GLY  ALA  THR  ALA  HIS  GLY  ALA  VAL
                                ...C G G C T T T G C C G G T G C A A C G G C G C A C G G T G C G G T
                                ... 2910              2920              2930              2940

  SER  VAL  GLY  ALA  SER  GLY  GLU  GLU  ARG...
T T C T G T C G G C G C A A G C G G C G A A G A A A G ...
             2950              2960              ...
                                ...  ARG  ILE  GLN  ASN  VAL  ALA  ALA  GLY  GLU  ILE  SER
                                ...A C G T A T C C A A A A C G T C G C G G C A G G C G A A A T T T C
                                ... 2970              2980              2990              3000

  ALA  THR  SER  THR  ASP  ALA  ILE  ASN  GLY...
C G C C A C T T C C A C C G A T G C G A T T A A C G G ...
             3010              3020              ...
                                ...  SER  GLN  LEU  TYR  ALA  VAL  ALA  LYS  GLY  VAL  THR
                                ...C A G C C A G T T G T A T G C T G T G G C A A A A G G G G T A A C
                                ... 3030              3040              3050              3060

  ASN  LEU  ALA  GLY  GLN  VAL  ASN  LYS  VAL...
A A A T C T T G C T G G A C A A G T G A A T A A A G T ...
             3070              3080              ...
                                ...  GLY  LYS  ARG  ALA  ASP  ALA  GLY  THR  ALA  SER  ALA
                                ...G G G C A A A C G T G C A G A T G C A G G T A C A G C A A G T G C
                                ... 3090              3100              3110              3120
```

FIG.23P

```
   LEU  ALA  ALA  SER  GLN  LEU  PRO  GLN  ALA...
A T T A G C A G C T T C A C A G T T A C C A C A A G C ...
            3130              3140              ...
                                ...  SER  MET  PRO  GLY  LYS  SER  MET  VAL  SER  ILE  ALA
                                ...C T C T A T G C C A G G T A A A T C A A T G G T T T C T A T T G C
                                ... 3150              3160              3170              3180

   GLY  SER  SER  TYR  GLN  GLY  GLN  ASN  GLY...
G G G A A G T A G T T A T C A A G G T C A A A A T G G ...
            3190              3200              ...
                                ...  LEU  ALA  ILE  GLY  VAL  SER  ARG  ILE  SER  ASP  ASN
                                ...T T T A G C T A T C G G G G T A T C A C G A A T T T C C G A T A A
                                ... 3210              3220              3230              3240

   GLY  LYS  VAL  ILE  ILE  ARG  LEU  SER  GLY...
T G G C A A A G T G A T T A T T C G C T T G T C A G G ...
            3250              3260              ...
                                ...  THR  THR  ASN  SER  GLN  GLY  LYS  THR  GLY  VAL  ALA
                                ...C A C A A C C A A T A G C C A A G G T A A A A C A G G C G T T G C
                                ... 3270              3280              3290              3300

   ALA  GLY  VAL  GLY  TYR  GLN  TRP  ***
A G C A G G T G T T G G T T A C C A G T G G T A A T A ...
            3310              3320              ...

                                ...G A A T T G A T C C G C
                                ... 3330
```

FIG.24A

H. influenzae type c strain API *hia* sequence

```
 MET  ASN  LYS  ILE  PHE  ASN  VAL  ILE  TRP  ASN ...
A T G A A C A A A A T T T T T A A C G T T A T T T G G A A T...
              10                   20                   30...
                                  ... VAL  MET  THR  GLN  THR  TRP  VAL  VAL  VAL  SER
                                  ... G T T A T G A C T C A A A C T T G G G T T G T C G T A T C T
                                  ...                  40                   50                   60

 GLU  LEU  THR  ARG  THR  HIS  THR  LYS  ARG  ALA ...
G A A C T C A C T C G C A C C C A C A C C A A A C G C G C C...
              70                   80                   90...
                                  ... SER  ALA  THR  VAL  GLU  THR  ALA  VAL  LEU  ALA
                                  ... T C C G C A A C C G T G G A G A C C G C C G T A T T G G C G
                                  ...                 100                  110                  120

 THR  LEU  LEU  PHE  ALA  THR  VAL  GLN  ALA  ASN ...
A C A C T G T T G T T T G C A A C G G T T C A G G C G A A T...
              130                  140                  150...
                                  ... ALA  THR  ASP  GLU  ASP  GLU  GLU  LEU  ASP  PRO
                                  ... G C T A C C G A T G A A G A T G A A G A G T T A G A C C C C
                                  ...                 160                  170                  180

 VAL  VAL  ARG  THR  ALA  PRO  VAL  LEU  SER  PHE ...
G T A G T A C G C A C T G C T C C C G T G T T G A G C T T C...
              190                  200                  210...
```

FIG.24B

```
                                    ... HIS  SER  ASP  LYS  GLU  GLY  THR  GLY  GLU  LYS
                                    ... C A T T C C G A T A A A G A A G G C A C G G G A G A A A A A
                                    ...              220                  230                  240

 GLU  VAL  THR  GLU  ASN  SER  ASN  TRP  GLY  ILE ...
G A A G T T A C A G A A A A T T C A A A T T G G G G A A T A...
              250                  260                  270...
                                    ... TYR  PHE  HIS  ASN  LYS  GLY  VAL  LEU  LYS  ALA
                                    ... T A T T T C C A C A A T A A A G G A G T A C T A A A A G C C
                                    ...              280                  290                  300

 GLY  ALA  ILE  THR  LEU  LYS  ALA  GLY  ASP  ASN ...
G G A G C A A T C A C C C T C A A A G C C G G C G A C A A C...
              310                  320                  330...
                                    ... LEU  LYS  ILE  LYS  GLN  SER  THR  ASN  ALA  SER
                                    ... C T G A A A A T C A A A C A A A G C A C C A A T G C C A G T
                                    ...              340                  350                  360

 SER  PHE  THR  TYR  SER  LEU  LYS  LYS  ASP  LEU ...
A G C T T C A C C T A C T C G C T G A A A A A A G A C C T C...
              370                  380                  390...
                                    ... THR  ASP  LEU  THR  SER  VAL  ALA  THR  GLU  LYS
                                    ... A C A G A T C T G A C C A G T G T T G C A A C T G A A A A A
                                    ...              400                  410                  420
```

FIG.24C

```
 LEU  SER  PHE  GLY  ALA  ASN  GLY  ASP  LYS  VAL ...
T T A T C G T T T G G C G C A A A C G G C G A T A A A G T T...
              430                  440                  450...
                                  ... ASP  ILE  THR  SER  ASP  ALA  ASN  GLY  LEU  LYS
                                  ... G A T A T T A C C A G T G A T G C A A A T G G C T T G A A A
                                  ...                  460                  470                  480

 LEU  ALA  LYS  THR  GLY  ASN  GLY  ASN  VAL  HIS ...
T T G G C G A A A A C A G G T A A C G G A A A T G T T C A T...
              490                  500                  510...
                                  ... LEU  ASN  GLY  LEU  ASP  SER  THR  LEU  PRO  ASP
                                  ... T T G A A T G G T T T G G A T T C A A C T T T G C C T G A T
                                  ...                  520                  530                  540

 ALA  VAL  THR  ASN  THR  GLY  VAL  LEU  SER  SER ...
G C G G T A A C G A A T A C A G G T G T G T T A A G T T C A...
              550                  560                  570...
                                  ... SER  SER  PHE  THR  PRO  ASN  ASP  VAL  GLU  LYS
                                  ... T C A A G T T T T A C A C C T A A T G A T G T T G A A A A A
                                  ...                  580                  590                  600

 THR  ARG  ALA  ALA  THR  VAL  LYS  ASP  VAL  LEU ...
A C A A G A G C T G C A A C T G T T A A A G A T G T T T T A...
              610                  620                  630...
```

FIG.24D

```
                                    ... ASN  ALA  GLY  TRP  ASN  ILE  LYS  GLY  ALA  LYS
                                    ... A A T G C A G G T T G G A A C A T T A A A G G T G C T A A A
                                    ...              640                650                660

 THR  ALA  GLY  GLY  ASN  VAL  GLU  SER  VAL  ASP ...
A C T G C T G G A G G T A A T G T T G A G A G T G T T G A T...
              670                680                690...
                                    ... LEU  VAL  SER  ALA  TYR  ASN  ASN  VAL  GLU  PHE
                                    ... T T A G T G T C C G C T T A T A A T A A T G T T G A A T T T
                                    ...              700                710                720

 ILE  THR  GLY  ASP  LYS  ASN  THR  LEU  ASP  VAL ...
A T T A C A G G C G A T A A A A A C A C G C T T G A T G T T...
              730                740                750...
                                    ... VAL  LEU  THR  ALA  LYS  GLU  ASN  GLY  LYS  THR
                                    ... G T A T T A A C A G C T A A A G A A A A C G G T A A A A C A
                                    ...              760                770                780

 THR  GLU  VAL  LYS  PHE  THR  PRO  LYS  THR  SER ...
A C C G A A G T G A A A T T C A C A C C G A A A A C C T C T...
              790                800                810...
                                    ... VAL  ILE  LYS  GLU  LYS  ASP  GLY  LYS  LEU  PHE
                                    ... G T T A T C A A A G A A A A A G A C G G T A A G T T A T T T
                                    ...              820                830                840
```

FIG.24E

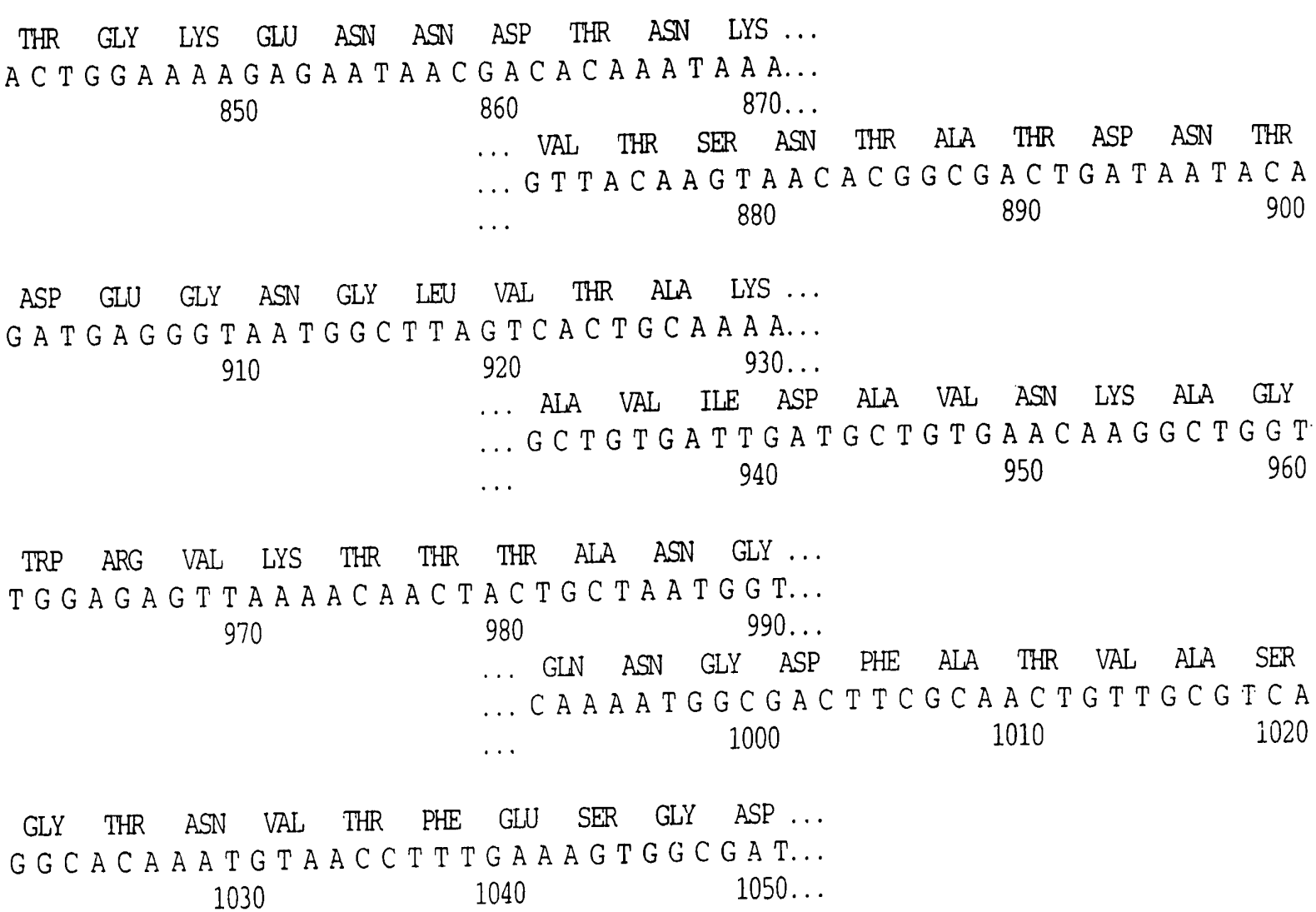


FIG.24F

```
... GLY THR THR ALA SER VAL THR LYS ASP THR
... G G T A C A A C A G C G T C A G T A A C T A A A G A T A C T
...              1060          1070          1080

ASN GLY ASN GLY ILE THR VAL LYS TYR ASP ...
A A C G G C A A T G G C A T C A C T G T T A A G T A C G A C...
          1090          1100          1110...
... ALA LYS VAL GLY ASP GLY LEU LYS PHE ASP
... G C G A A A G T T G G C G A C G G C T T G A A A T T T G A T
...              1120          1130          1140

SER ASP LYS LYS ILE VAL ALA ASP THR THR ...
A G C G A T A A A A A A A T C G T T G C A G A T A C G A C C...
          1150          1160          1170...
... ALA LEU THR VAL THR GLY GLY LYS VAL ALA
... G C A C T T A C T G T G A C A G G T G G T A A G G T A G C T
...              1180          1190          1200

GLU ILE ALA LYS GLU ASP ASP LYS LYS LYS ...
G A A A T T G C T A A A G A A G A T G A C A A G A A A A A A...
          1210          1220          1230...
... LEU VAL ASN ALA GLY ASP LEU VAL THR ALA
... C T T G T T A A T G C A G G C G A T T T G G T A A C A G C T
...              1240          1250          1260
```

FIG.24G

```
 LEU  GLY  ASN  LEU  SER  TRP  LYS  ALA  LYS  ALA ...
T T A G G T A A T C T A A G T T G G A A A G C A A A A G C T...
          1270           1280           1290...
                              ... GLU  ALA  ASP  THR  ASP  THR  ASP  GLY  ALA  LEU
                              ... G A G G C T G A T A C T G A T A C T G A T G G T G C G C T T
                              ...          1300           1310           1320

 GLU  GLY  ILE  SER  LYS  ASP  GLN  GLU  VAL  LYS ...
G A G G G G A T T T C A A A A G A C C A A G A A G T C A A A...
          1330           1340           1350...
                              ... ALA  GLY  GLU  THR  VAL  THR  PHE  LYS  ALA  GLY
                              ... G C A G G C G A A A C G G T A A C C T T T A A A G C G G G C
                              ...          1360           1370           1380

 LYS  ASN  LEU  LYS  VAL  LYS  GLN  ASP  GLY  ALA ...
A A G A A C T T A A A A G T G A A A C A G G A T G G T G C G...
          1390           1400           1410...
                              ... ASN  PHE  THR  TYR  SER  LEU  GLN  ASP  ALA  LEU
                              ... A A C T T T A C T T A T T C A C T G C A A G A T G C T T T A
                              ...          1420           1430           1440

 THR  GLY  LEU  THR  SER  ILE  THR  LEU  GLY  GLY ...
A C G G G T T T A A C G A G C A T T A C T T T A G G T G G T...
          1450           1460           1470...
```

FIG.24H

```
                                    ... THR  THR  ASN  GLY  GLY  ASN  ASP  ALA  LYS  THR
                                    ... A C A A C T A A T G G C G G A A A T G A T G C G A A A A C C
                                    ...                1480              1490              1500

 VAL  ILE  ASN  LYS  ASP  GLY  LEU  THR  ILE  THR ...
G T C A T C A A C A A A G A C G G T T T A A C C A T C A C G...
              1510              1520              1530...
                                    ... PRO  ALA  GLY  ASN  GLY  GLY  THR  THR  GLY  THR
                                    ... C C A G C A G G T A A T G G C G G T A C G A C A G G T A C A
                                    ...                1540              1550              1560

 ASN  THR  ILE  SER  VAL  THR  LYS  ASP  GLY  ILE ...
A A C A C C A T C A G C G T A A C C A A A G A T G G C A T T...
              1570              1580              1590...
                                    ... LYS  ALA  GLY  ASN  LYS  ALA  ILE  THR  ASN  VAL
                                    ... A A A G C A G G T A A T A A A G C T A T T A C T A A T G T T
                                    ...                1600              1610              1620

 ALA  SER  GLY  LEU  ARG  ALA  TYR  ASP  ASP  ALA ...
G C G A G T G G T T T A A G A G C T T A T G A C G A T G C G...
              1630              1640              1650...
                                    ... ASN  PHE  ASP  VAL  LEU  ASN  ASN  SER  ALA  THR
                                    ... A A T T T T G A T G T T T T A A A T A A C T C T G C A A C T
                                    ...                1660              1670              1680
```

FIG.24I

```
 ASP  LEU  ASN  ARG  HIS  VAL  GLU  ASP  ALA  TYR ...
G A T T T A A A T A G A C A C G T T G A A G A T G C T T A T...
            1690              1700              1710...
                              ... LYS  GLY  LEU  LEU  ASN  LEU  ASN  GLU  LYS  ASN
                              ... A A A G G T T T A T T A A A T C T A A A T G A A A A A A A T
                              ...            1720              1730              1740

 ALA  ASN  LYS  GLN  PRO  LEU  VAL  THR  ASP  SER ...
G C A A A T A A A C A A C C G T T G G T G A C T G A C A G C...
            1750              1760              1770...
                              ... THR  ALA  ALA  THR  VAL  GLY  ASP  LEU  ARG  LYS
                              ... A C G G C G G C G A C T G T A G G C G A T T T A C G T A A A
                              ...            1780              1790              1800

 LEU  GLY  TRP  VAL  VAL  SER  THR  LYS  ASN  GLY ...
T T G G G T T G G G T A G T A T C A A C C A A A A A C G G T...
            1810              1820              1830...
                              ... THR  LYS  GLU  GLU  SER  ASN  GLN  VAL  LYS  GLN
                              ... A C G A A A G A A G A A A G C A A T C A A G T T A A A C A A
                              ...            1840              1850              1860

 ALA  ASP  GLU  VAL  LEU  PHE  THR  GLY  ALA  GLY ...
G C T G A T G A A G T C C T C T T T A C C G G A G C C G G T...
            1870              1880              1890...
```

FIG.24J

```
                                    ... ALA  ALA  THR  VAL  THR  SER  LYS  SER  GLU  ASN
                                    ... G C T G C T A C G G T T A C T T C C A A A T C T G A A A A C
                                    ...              1900              1910              1920

 GLY  LYS  HIS  THR  ILE  THR  VAL  SER  VAL  ALA ...
G G T A A A C A T A C G A T T A C C G T T A G T G T G G C T...
              1930              1940              1950...
                                    ... GLU  THR  LYS  ALA  ASP  SER  GLY  LEU  GLU  LYS
                                    ... G A A A C T A A A G C G G A T A G C G G T C T T G A A A A A
                                    ...              1960              1970              1980

 ASP  GLY  ASP  THR  ILE  LYS  LEU  LYS  VAL  ASP ...
G A T G G C G A T A C T A T T A A G C T C A A A G T G G A T...
              1990              2000              2010...
                                    ... ASN  GLN  ASN  THR  ASP  ASN  VAL  LEU  THR  VAL
                                    ... A A T C A A A A C A C T G A T A A T G T T T T A A C T G T T
                                    ...              2020              2030              2040

 GLY  ASN  ASN  GLY  THR  ALA  VAL  THR  LYS  GLY ...
G G T A A T A A T G G T A C T G C T G T C A C T A A A G G T...
              2050              2060              2070...
                                    ... GLY  PHE  GLU  THR  VAL  LYS  THR  GLY  ALA  THR
                                    ... G G C T T T G A A A C T G T T A A A A C T G G A G C G A C T
                                    ...              2080              2090              2100
```

FIG.24K

```
 ASP   ALA   ASP   ARG   GLY   LYS   VAL   THR   VAL   LYS ...
G A T G C A G A T C G C G G T A A A G T A A C T G T A A A A...
             2110                2120                2130...
                                 ... ASP   ALA   THR   ALA   ASN   ASP   ALA   ASP   LYS   LYS
                                 ... G A T G C T A C T G C T A A T G A C G C T G A T A A G A A A
                                 ...                 2140                2150                2160

 VAL   ALA   THR   VAL   LYS   ASP   VAL   ALA   THR   ALA ...
G T C G C A A C T G T A A A A G A T G T T G C A A C C G C A...
             2170                2180                2190...
                                 ... ILE   ASN   SER   ALA   ALA   THR   PHE   VAL   LYS   THR
                                 ... A T T A A T A G T G C G G C G A C T T T T G T G A A A A C A
                                 ...                 2200                2210                2220

 GLU   ASN   LEU   THR   THR   SER   ILE   ASP   GLU   ASP ...
G A G A A T T T A A C T A C C T C T A T T G A T G A A G A T...
             2230                2240                2250...
                                 ... ASN   PRO   THR   ASP   ASN   GLY   LYS   ASP   ASP   ALA
                                 ... A A T C C T A C A G A T A A C G G C A A A G A T G A C G C A
                                 ...                 2260                2270                2280

 LEU   LYS   ALA   GLY   ASP   THR   LEU   THR   PHE   LYS ...
C T T A A A G C G G G C G A T A C C T T A A C C T T T A A A...
             2290                2300                2310...
```

FIG.24L

```
                                    ... ALA GLY LYS ASN LEU LYS VAL LYS ARG ASP
                                    ... G C A G G T A A A A A C C T G A A A G T T A A A C G T G A T
                                    ...          2320          2330          2340

 GLY LYS ASN ILE THR PHE ASP LEU ALA LYS ...
G G A A A A A A T A T T A C T T T T G A C T T G G C G A A A...
          2350          2360          2370...
                                    ... ASN LEU GLU VAL LYS THR ALA LYS VAL SER
                                    ... A A C C T T G A G G T G A A A A C T G C G A A A G T G A G T
                                    ...          2380          2390          2400

 ASP THR LEU THR ILE GLY GLY ASN THR PRO ...
G A T A C T T T A A C G A T T G G C G G G A A T A C A C C T...
          2410          2420          2430...
                                    ... THR GLY GLY THR THR ALA THR PRO LYS VAL
                                    ... A C A G G T G G C A C T A C T G C G A C G C C A A A A G T G
                                    ...          2440          2450          2460

 ASN ILE THR SER THR ALA ASP GLY LEU ASN ...
A A T A T T A C T A G C A C G G C T G A T G G T T T G A A T...
          2470          2480          2490...
                                    ... PHE ALA LYS GLU THR ALA ASP ALA SER GLY
                                    ... T T T G C A A A A G A A A C A G C C G A T G C C T C G G G T
                                    ...          2500          2510          2520
```

FIG.24M

```
 SER  LYS  ASN  VAL  TYR  LEU  LYS  GLY  ILE  ALA ...
T C T A A G A A T G T T T A T T T G A A A G G T A T T G C G...
           2530              2540              2550...
                                ... THR  THR  LEU  THR  GLU  PRO  SER  ALA  GLY  ALA
                                ... A C A A C T T T A A C T G A G C C A A G C G C G G G A G C G
                                ...            2560              2570              2580

 LYS  SER  SER  HIS  VAL  ASP  LEU  ASN  VAL  ASP ...
A A G T C T T C A C A C G T T G A T T T A A A T G T G G A T...
           2590              2600              2610...
                                ... ALA  THR  LYS  LYS  SER  ASN  ALA  ALA  SER  ILE
                                ... G C G A C G A A A A A A T C C A A T G C A G C A A G T A T T
                                ...            2620              2630              2640

 GLU  ASP  VAL  LEU  ARG  ALA  GLY  TRP  ASN  ILE ...
G A A G A T G T A T T G C G C G C A G G T T G G A A T A T T...
           2650              2660              2670...
                                ... GLN  GLY  ASN  GLY  ASN  ASN  VAL  ASP  TYR  VAL
                                ... C A A G G T A A T G G T A A T A A T G T T G A T T A T G T A
                                ...            2680              2690              2700

 ALA  THR  TYR  ASP  THR  VAL  ASN  PHE  THR  ASP ...
G C G A C G T A T G A C A C A G T A A A C T T T A C C G A T...
           2710              2720              2730...
```

FIG.24N

```
... ASP SER THR GLY THR THR THR VAL THR VAL
... G A C A G C A C A G G T A C A A C A A C G G T A A C C G T A
...              2740              2750              2760

 THR GLN LYS ALA ASP GLY LYS GLY ALA ASP ...
A C C C A A A A A G C A G A T G G C A A A G G T G C T G A C...
             2770              2780              2790...
                  ... VAL LYS ILE GLY ALA LYS THR SER VAL ILE
                  ... G T T A A A A T C G G T G C G A A A A C T T C T G T T A T C
                  ...              2800              2810              2820

 LYS ASP HIS ASN GLY LYS LEU PHE THR GLY ...
A A A G A C C A C A A C G G C A A A C T G T T T A C A G G C...
             2830              2840              2850...
                  ... LYS ASP LEU LYS ASP ALA ASN ASN GLY ALA
                  ... A A A G A C C T G A A A G A T G C G A A T A A T G G T G C A
                  ...              2860              2870              2880

 THR VAL SER GLU ASP ASP GLY LYS ASP THR ...
A C C G T T A G T G A A G A T G A T G G C A A A G A C A C C...
             2890              2900              2910...
                  ... GLY THR GLY LEU VAL THR ALA LYS THR VAL
                  ... G G C A C A G G C T T A G T T A C T G C A A A A A C T G T G
                  ...              2920              2930              2940
```

FIG.24O

```
 ILE  ASP  ALA  VAL  ASN  LYS  SER  GLY  TRP  ARG ...
A T T G A T G C A G T A A A T A A A A G C G G T T G G A G G...
             2950              2960              2970...
                                ... VAL  THR  GLY  GLU  GLY  ALA  THR  ALA  GLU  THR
                                ... G T A A C C G G T G A G G G C G C G A C T G C C G A A A C C
                                ...              2980              2990              3000

 GLY  ALA  THR  ALA  VAL  ASN  ALA  GLY  ASN  ALA ...
G G T G C A A C C G C C G T G A A T G C G G G T A A C G C T...
             3010              3020              3030...
                                ... GLU  THR  VAL  THR  SER  GLY  THR  SER  VAL  ASN
                                ... G A A A C C G T T A C A T C A G G C A C G A G C G T G A A C
                                ...              3040              3050              3060

 PHE  LYS  ASN  GLY  ASN  ALA  THR  THR  ALA  THR ...
T T C A A A A A C G G C A A T G C G A C C A C A G C G A C C...
             3070              3080              3090...
                                ... VAL  SER  LYS  ASP  ASN  GLY  ASN  ILE  ASN  VAL
                                ... G T A A G C A A A G A T A A T G G C A A C A T C A A T G T C
                                ...              3100              3110              3120

 LYS  TYR  ASP  VAL  ASN  VAL  GLY  ASP  GLY  LEU ...
A A A T A C G A T G T A A A T G T T G G T G A C G G C T T G...
             3130              3140              3150...
```

FIG.24P

```
... LYS ILE GLY ASP ASP LYS LYS ILE VAL ALA
... A A G A T T G G C G A T G A C A A A A A A A T C G T T G C A
...          3160          3170          3180

 ASP THR THR THR LEU THR VAL THR GLY GLY ...
G A C A C G A C C A C A C T T A C T G T A A C A G G T G G T...
          3190          3200          3210...
                                ... LYS VAL SER VAL PRO ALA GLY ALA ASN SER
                                ... A A G G T G T C T G T T C C T G C T G G T G C T A A T A G T
                                ...          3220          3230          3240

 VAL ASN ASN ASN LYS LYS LEU VAL ASN ALA ...
G T T A A T A A C A A T A A G A A A C T T G T T A A T G C A...
          3250          3260          3270...
                                ... GLU GLY LEU ALA THR ALA LEU ASN ASN LEU
                                ... G A G G G T T T A G C G A C T G C T T T A A A C A A C C T A
                                ...          3280          3290          3300

 SER TRP THR ALA LYS ALA ASP LYS TYR ALA ...
A G C T G G A C G G C A A A A G C C G A T A A A T A T G C A...
          3310          3320          3330...
                                ... ASP GLY GLU SER GLU GLY GLU THR ASP GLN
                                ... G A T G G C G A G T C A G A G G G C G A A A C C G A C C A A
                                ...          3340          3350          3360
```

FIG.24Q

```
 GLU  VAL  LYS  ALA  GLY  ASP  LYS  VAL  THR  PHE ...
G A A G T C A A A G C A G G C G A C A A A G T A A C C T T T...
          3370                 3380                 3390....

...  LYS  ALA  GLY  LYS  ASN  LEU  LYS  VAL  LYS  GLN
... A A A G C A G G C A A G A A C T T A A A A G T G A A A C A G
          3400                 3410                 3420
...

 SER  GLU  LYS  ASP  PHE  THR  TYR  SER  LEU  GLN ...
T C T G A A A A A G A C T T T A C T T A T T C A C T G C A A...
          3430                 3440                 3450....

...  ASP  THR  LEU  THR  GLY  LEU  THR  SER  ILE  THR
... G A C A C T T T A A C A G G C T T A A C G A G C A T T A C T
          3460                 3470                 3480
...

 LEU  GLY  GLY  THR  ALA  ASN  GLY  ARG  ASN  ASP ...
T T A G G T G G T A C A G C T A A T G G C A G A A A T G A T...
          3490                 3500                 3510....

...  THR  GLY  THR  VAL  ILE  ASN  LYS  ASP  GLY  LEU
... A C G G G A A C C G T C A T C A A C A A A G A C G G C T T A
          3520                 3530                 3540
...

 THR  ILE  THR  LEU  ALA  ASN  GLY  ALA  ALA  ALA ...
A C C A T C A C G C T G G C A A A T G G T G C T G C G G C A...
          3550                 3560                 3570....
```

FIG.24R

```
                                  ... GLY  THR  ASP  ALA  SER  ASN  GLY  ASN  THR  ILE
                                  ... G G C A C A G A T G C G T C T A A C G G A A A C A C C A T C
                                  ...              3580              3590              3600

 SER  VAL  THR  LYS  ASP  GLY  ILE  SER  ALA  GLY ...
A G T G T A A C C A A A G A C G G C A T T A G T G C G G G T...
            3610              3620              3630...
                                  ... ASN  LYS  GLU  ILE  THR  ASN  VAL  LYS  SER  ALA
                                  ... A A T A A A G A A A T T A C C A A T G T T A A G A G T G C T
                                  ...              3640              3650              3660

 LEU  LYS  THR  TYR  LYS  ASP  THR  GLN  ASN  THR ...
T T A A A A A C C T A T A A A G A T A C T C A A A A C A C T...
            3670              3680              3690...
                                  ... ALA  GLY  ALA  THR  GLN  PRO  ALA  ALA  ASN  THR
                                  ... G C A G G T G C A A C T C A A C C T G C G G C T A A T A C A
                                  ...              3700              3710              3720

 ALA  GLU  VAL  ALA  LYS  GLN  ASP  LEU  VAL  ASP ...
G C T G A A G T A G C C A A A C A A G A C T T G G T T G A T...
            3730              3740              3750...
                                  ... LEU  THR  LYS  PRO  ALA  THR  GLY  ALA  ALA  GLY
                                  ... T T A A C T A A A C C T G C G A C A G G T G C A G C T G G A
                                  ...              3760              3770              3780
```

FIG.24S

```
ASN GLY ALA ASP ALA LYS ALA PRO ASP THR ...
AATGGTGCAGATGCAAAAGCTCCCGATACC...
        3790            3800            3810...
                                ... THR ALA ALA THR VAL GLY ASP LEU ARG GLY
                                ... ACAGCTGCAACCGTAGGCGACTTGCGTGGT
                                ...         3820            3830            3840

LEU GLY TRP VAL LEU SER ALA LYS LYS THR ...
TTGGGCTGGGTGCTTTCAGCTAAGAAAACT...
        3850            3860            3870...
                                ... ALA ASP GLU THR GLN ASP LYS GLU PHE HIS
                                ... GCAGATGAAACACAAGATAAAGAGTTCCAC
                                ...         3880            3890            3900

ALA ALA VAL LYS ASN ALA ASN GLU VAL GLU ...
GCCGCCGTTAAAAACGCAAATGAAGTTGAG...
        3910            3920            3930...
                                ... PHE VAL GLY LYS ASN GLY ALA THR VAL SER
                                ... TTCGTGGGTAAAAACGGTGCAACCGTGTCT
                                ...         3940            3950            3960

ALA LYS THR ASP ASN ASN GLY LYS HIS THR ...
GCAAAAACTGATAACAACGGAAAACATACT...
        3970            3980            3990...
```

FIG.24T

```
                                    ... VAL  THR  ILE  ASP  VAL  ALA  GLU  ALA  LYS  VAL
                                    ... G T A A C G A T T G A T G T T G C A G A A G C C A A A G T T
                                    ...              4000              4010              4020

 GLY  ASP  GLY  LEU  GLU  LYS  ASP  THR  ASP  GLY ...
G G T G A T G G T C T T G A A A A A G A T A C T G A C G G C...
              4030              4040              4050...
                                    ... LYS  ILE  LYS  LEU  LYS  VAL  ASP  ASN  THR  ASP
                                    ... A A G A T T A A A C T C A A A G T A G A T A A T A C A G A T
                                    ...              4060              4070              4080

 GLY  ASN  ASN  LEU  LEU  THR  VAL  ASP  ALA  THR ...
G G G A A T A A T C T A T T A A C C G T T G A T G C A A C A...
              4090              4100              4110...
                                    ... LYS  GLY  ALA  SER  VAL  ALA  LYS  GLY  GLU  PHE
                                    ... A A A G G T G C A T C C G T T G C C A A G G G C G A G T T T
                                    ...              4120              4130              4140

 ASN  ALA  VAL  THR  THR  ASP  ALA  THR  THR  ALA ...
A A T G C C G T A A C A A C A G A T G C A A C T A C A G C C...
              4150              4160              4170...
                                    ... GLN  GLY  THR  ASN  ALA  ASN  GLU  ARG  GLY  LYS
                                    ... C A A G G C A C A A A T G C C A A T G A G C G C G G T A A A
                                    ...              4180              4190              4200
```

FIG.24U

```
 VAL  VAL  VAL  LYS  GLY  SER  ASN  GLY  ALA  THR ...
G T G G T T G T C A A G G G T T C A A A T G G T G C A A C T...
              4210                 4220                 4230...
                                    ... ALA  THR  GLU  THR  ASP  LYS  LYS  LYS  VAL  ALA
                                    ... G C T A C C G A A A C T G A C A A G A A A A A A G T G G C A
                                    ...                 4240                 4250                 4260

 THR  VAL  GLY  ASP  VAL  ALA  LYS  ALA  ILE  ASN ...
A C T G T T G G C G A C G T T G C T A A A G C G A T T A A C...
              4270                 4280                 4290...
                                    ... ASP  ALA  ALA  THR  PHE  VAL  LYS  VAL  GLU  ASN
                                    ... G A C G C A G C A A C T T T C G T G A A A G T G G A A A A T
                                    ...                 4300                 4310                 4320

 ASP  ASP  SER  ALA  THR  ILE  ASP  ASP  SER  PRO ...
G A C G A C A G T G C T A C G A T T G A T G A T A G C C C A...
              4330                 4340                 4350...
                                    ... THR  ASP  ASP  GLY  ALA  ASN  ASP  ALA  LEU  LYS
                                    ... A C A G A T G A T G G C G C A A A T G A T G C T C T C A A A
                                    ...                 4360                 4370                 4380

 ALA  GLY  ASP  THR  LEU  THR  LEU  LYS  ALA  GLY ...
G C A G G C G A C A C C T T G A C C T T A A A A G C G G G T...
              4390                 4400                 4410...
```

FIG.24V

```
... LYS ASN LEU LYS VAL LYS ARG ASP GLY LYS
... A A A A A C T T A A A A G T T A A A C G T G A T G G T A A A
...              4420              4430              4440

 ASN ILE THR PHE ALA LEU ALA ASN ASP LEU ...
A A T A T T A C T T T T G C C C T T G C G A A C G A C C T T...
            4450              4460              4470...
                ... SER VAL LYS SER ALA THR VAL SER ASP LYS
                ... A G T G T A A A A A G C G C A A C C G T T A G C G A T A A A
                ...              4480              4490              4500

 LEU SER LEU GLY THR ASN GLY ASN LYS VAL ...
T T A T C G C T T G G T A C A A A C G G C A A T A A A G T C...
            4510              4520              4530...
                ... ASN ILE THR SER ASP THR LYS GLY LEU ASN
                ... A A T A T C A C A A G C G A C A C C A A A G G C T T G A A C
                ...              4540              4550              4560

 PHE ALA LYS ASP SER LYS THR GLY ASP ASP ...
T T C G C T A A A G A T A G T A A G A C A G G C G A T G A T...
            4570              4580              4590...
                ... ALA ASN ILE HIS LEU ASN GLY ILE ALA SER
                ... G C T A A T A T T C A C T T A A A T G G C A T T G C T T C A
                ...              4600              4610              4620
```

FIG.24W

```
 THR  LEU  THR  ASP  THR  LEU  LEU  ASN  SER  GLY ...
A C T T T A A C T G A T A C A T T G T T A A A T A G T G G T...
          4630              4640              4650...
                            ... ALA  THR  THR  ASN  LEU  GLY  GLY  ASN  GLY  ILE
                            ... G C G A C A A C C A A T T T A G G T G G T A A T G G T A T T
                            ...           4660              4670              4680

 THR  ASP  ASN  GLU  LYS  LYS  ARG  ALA  ALA  SER ...
A C T G A T A A C G A G A A A A A A C G C G C G G C G A G C...
          4690              4700              4710...
                            ... VAL  LYS  ASP  VAL  LEU  ASN  ALA  GLY  TRP  ASN
                            ... G T T A A A G A T G T C T T G A A T G C G G G T T G G A A T
                            ...           4720              4730              4740

 VAL  ARG  GLY  VAL  LYS  PRO  ALA  SER  ALA  ASN ...
G T T C G T G G T G T T A A A C C G G C A T C T G C A A A T...
          4750              4760              4770...
                            ... ASN  GLN  VAL  GLU  ASN  ILE  ASP  PHE  VAL  ALA
                            ... A A T C A A G T G G A G A A T A T C G A C T T T G T A G C A
                            ...           4780              4790              4800

 THR  TYR  ASP  THR  VAL  ASP  PHE  VAL  SER  GLY ...
A C C T A C G A C A C A G T G G A C T T T G T T A G T G G A...
          4810              4820              4830...
```

FIG.24X

... ASP LYS ASP THR THR SER VAL THR VAL GLU
... G A T A A A G A C A C C A C G A G T G T A A C T G T T G A A
... 4840 4850 4860

SER LYS ASP ASN GLY LYS ARG THR GLU VAL ...
A G T A A A G A T A A T G G C A A G A G A A C C G A A G T T...
4870 4880 4890...

... LYS ILE GLY ALA LYS THR SER VAL ILE LYS
... A A A A T C G G T G C G A A G A C T T C T G T T A T C A A A
... 4900 4910 4920

ASP HIS ASN GLY LYS LEU PHE THR GLY LYS ...
G A C C A C A A C G G C A A A C T G T T T A C A G G C A A A...
4930 4940 4950...

... GLU LEU LYS ASP ALA ASN ASN ASN GLY VAL
... G A G C T G A A G G A T G C T A A C A A T A A T G G C G T A
... 4960 4970 4980

THR VAL THR GLU THR ASP GLY LYS ASP GLU ...
A C T G T T A C C G A A A C C G A C G G C A A A G A C G A G...
4990 5000 5010...

... GLY ASN GLY LEU VAL THR ALA LYS ALA VAL
... G G T A A T G G T T T A G T G A C T G C A A A A G C T G T G
... 5020 5030 5040

FIG.24Y

```
 ILE  ASP  ALA  VAL  ASN  LYS  ALA  GLY  TRP  ARG ...
A T T G A T G C C G T G A A T A A G G C T G G T T G G A G A...
          5050              5060              5070...
                                ... VAL  LYS  THR  THR  GLY  ALA  ASN  GLY  GLN  ASN
                                ... G T T A A A A C A A C A G G T G C T A A T G G T C A G A A T
                                ...           5080              5090              5100

 ASP  ASP  PHE  ALA  THR  VAL  ALA  SER  GLY  THR ...
G A T G A C T T C G C A A C T G T T G C G T C A G G C A C A...
          5110              5120              5130...
                                ... ASN  VAL  THR  PHE  ALA  ASP  GLY  ASN  GLY  THR
                                ... A A T G T A A C C T T T G C T G A T G G T A A T G G C A C A
                                ...           5140              5150              5160

 THR  ALA  GLU  VAL  THR  LYS  ALA  ASN  ASP  GLY ...
A C T G C C G A A G T A A C T A A A G C A A A C G A C G G T...
          5170              5180              5190...
                                ... SER  ILE  THR  VAL  LYS  TYR  ASN  VAL  LYS  VAL
                                ... A G T A T T A C T G T T A A A T A C A A T G T T A A A G T G
                                ...           5200              5210              5220

 ALA  ASP  GLY  LEU  LYS  LEU  ASP  GLY  ASP  LYS ...
G C T G A T G G C T T A A A A C T A G A C G G C G A T A A A...
          5230              5240              5250...
```

FIG.24Z

```
... ILE VAL ALA ASP THR THR VAL LEU THR VAL
... A T C G T T G C A G A C A C G A C C G T A C T T A C T G T G
...          5260          5270          5280

 ALA ASP GLY LYS VAL THR ALA PRO ASN ASN ...
G C A G A T G G T A A A G T T A C A G C T C C G A A T A A T...
          5290          5300          5310...
                                ... GLY ASP GLY LYS LYS PHE VAL ASP ALA SER
                                ... G G C G A T G G T A A G A A A T T T G T T G A T G C A A G T
                                ...          5320          5330          5340

 GLY LEU ALA ASP ALA LEU ASN LYS LEU SER ...
G G T T T A G C G G A T G C G T T A A A T A A A T T A A G C...
          5350          5360          5370...
                                ... TRP THR ALA THR ALA GLY LYS GLU GLY THR
                                ... T G G A C G G C A A C T G C T G G T A A A G A A G G C A C T
                                ...          5380          5390          5400

 GLY GLU VAL ASP PRO ALA ASN SER ALA GLY ...
G G T G A A G T T G A T C C T G C A A A T T C A G C A G G G...
          5410          5420          5430...
                                ... GLN GLU VAL LYS ALA GLY ASP LYS VAL THR
                                ... C A A G A A G T C A A A G C G G G C G A C A A A G T A A C C
                                ...          5440          5450          5460
```

FIG.24A'

```
 PHE   LYS   ALA   GLY   ASP   ASN   LEU   LYS   ILE   LYS ...
T T T A A A G C C G G C G A C A A C C T G A A A A T C A A A...
             5470                5480                5490...
                                 ... GLN   SER   GLY   LYS   ASP   PHE   THR   TYR   SER   LEU
                                 ... C A A A G C G G C A A A G A C T T T A C C T A C T C G C T G
                                 ...               5500                5510                5520

 LYS   LYS   GLU   LEU   LYS   ASP   LEU   THR   SER   VAL ...
A A A A A A G A G C T G A A A G A C C T G A C C A G C G T A...
             5530                5540                5550...
                                 ... GLU   PHE   LYS   ASP   ALA   ASN   GLY   GLY   THR   GLY
                                 ... G A G T T C A A A G A C G C A A A C G G C G G T A C A G G C
                                 ...               5560                5570                5580

 SER   GLU   SER   THR   LYS   ILE   THR   LYS   ASP   GLY ...
A G T G A A A G C A C C A A G A T T A C C A A A G A C G G C...
             5590                5600                5610...
                                 ... LEU   THR   ILE   THR   PRO   ALA   ASN   GLY   ALA   GLY
                                 ... T T G A C C A T T A C G C C G G C A A A C G G T G C G G G T
                                 ...               5620                5630                5640

 ALA   ALA   GLY   ALA   ASN   THR   ALA   ASN   THR   ILE ...
G C G G C A G G T G C A A A C A C T G C A A A C A C C A T T...
             5650                5660                5670...
```

FIG.24B'

```
                                    ... SER VAL THR LYS ASP GLY ILE SER ALA GLY
                                    ... A G C G T A A C C A A A G A T G G C A T T A G C G C G G G T
                                    ...              5680              5690              5700

 ASN LYS ALA VAL THR ASN VAL VAL SER GLY ...
A A T A A A G C A G T T A C A A A C G T T G T G A G C G G A...
            5710              5720              5730...
                                    ... LEU LYS LYS PHE GLY ASP GLY HIS THR LEU
                                    ... C T G A A G A A A T T T G G T G A T G G T C A T A C G T T G
                                    ...              5740              5750              5760

 ALA ASN GLY THR VAL ALA ASP PHE GLU LYS ...
G C A A A T G G C A C T G T T G C T G A T T T T G A A A A G...
            5770              5780              5790...
                                    ... HIS TYR ASP ASN ALA TYR LYS ASP LEU THR
                                    ... C A T T A T G A C A A T G C C T A T A A A G A C T T G A C C
                                    ...              5800              5810              5820

 ASN LEU ASP GLU LYS GLY ALA ASP ASN ASN ...
A A T T T G G A T G A A A A A G G C G C G G A T A A T A A T...
            5830              5840              5850...
                                    ... PRO THR VAL ALA ASP ASN THR ALA ALA THR
                                    ... C C G A C T G T T G C C G A C A A T A C C G C T G C A A C C
                                    ...              5860              5870              5880
```

FIG.24C'

```
 VAL   GLY   ASP   LEU   ARG   GLY   LEU   GLY   TRP   VAL ...
G T G G G C G A T T T G C G C G G C T T G G G C T G G G T C...
             5890                5900                5910...
                                  ...  ILE   SER   ALA   ASP   LYS   THR   THR   GLY   GLU   PRO
                                  ... A T T T C T G C G G A C A A A A C C A C A G G C G A A C C C
                                  ...               5920                5930                5940

 ASN   GLN   GLU   TYR   ASN   ALA   GLN   VAL   ARG   ASN ...
A A T C A G G A A T A C A A C G C G C A A G T G C G T A A C...
             5950                5960                5970...
                                  ...  ALA   ASN   GLU   VAL   LYS   PHE   LYS   SER   GLY   ASN
                                  ... G C C A A T G A A G T G A A A T T C A A G A G C G G C A A C
                                  ...               5980                5990                6000

 GLY   ILE   ASN   VAL   SER   GLY   LYS   THR   LEU   ASN ...
G G T A T C A A T G T T T C C G G T A A A A C A T T G A A C...
             6010                6020                6030...
                                  ...  GLY   THR   ARG   VAL   ILE   THR   PHE   GLU   LEU   ALA
                                  ... G G T A C G C G C G T G A T T A C C T T T G A A T T G G C T
                                  ...               6040                6050                6060

 LYS   GLY   GLU   VAL   VAL   LYS   SER   ASN   GLU   PHE ...
A A A G G C G A A G T G G T T A A A T C G A A T G A A T T T...
             6070                6080                6090...
```

FIG.24D'

```
                                    ... THR VAL LYS ASN ALA ASP GLY SER GLU THR
                                    ... A C C G T T A A G A A T G C C G A T G G T T C G G A A A C G
                                    ...          6100          6110          6120

 ASN LEU VAL LYS VAL GLY ASP MET TYR TYR ...
A A C T T G G T T A A A G T T G G C G A T A T G T A T T A C...
          6130          6140          6150...
                                    ... SER LYS GLU ASP ILE ASP PRO ALA THR SER
                                    ... A G C A A A G A G G A T A T T G A C C C G G C A A C C A G T
                                    ...          6160          6170          6180

 LYS PRO MET THR GLY LYS THR GLU LYS TYR ...
A A A C C G A T G A C A G G T A A A A C T G A A A A A T A T...
          6190          6200          6210...
                                    ... LYS VAL GLU ASN GLY LYS VAL VAL SER ALA
                                    ... A A G G T T G A A A A C G G C A A A G T C G T T T C T G C T
                                    ...          6220          6230          6240

 ASN GLY SER LYS THR GLU VAL THR LEU THR ...
A A C G G C A G C A A G A C C G A A G T T A C C C T A A C C...
          6250          6260          6270...
                                    ... ASN LYS GLY SER GLY TYR VAL THR GLY ASN
                                    ... A A C A A A G G T T C C G G C T A T G T A A C A G G T A A C
                                    ...          6280          6290          6300
```

FIG.24E'

```
 GLN  VAL  ALA  ASP  ALA  ILE  ALA  LYS  SER  GLY ...
C A A G T G G C T G A T G C G A T T G C G A A A T C A G G C...
          6310              6320              6330...
                                ... PHE  GLU  LEU  GLY  LEU  ALA  ASP  ALA  ALA  GLU
                                ... T T T G A G C T T G G T T T G G C T G A T G C G G C A G A A
                                ...           6340              6350              6360

 ALA  GLU  LYS  ALA  PHE  ALA  GLU  SER  ALA  LYS ...
G C T G A A A A A G C C T T T G C A G A A A G C G C A A A A...
          6370              6380              6390...
                                ... ASP  LYS  GLN  LEU  SER  LYS  ASP  LYS  ALA  GLU
                                ... G A C A A G C A A T T G T C T A A A G A T A A A G C G G A A
                                ...           6400              6410              6420

 THR  VAL  ASN  ALA  HIS  ASP  LYS  VAL  ARG  PHE ...
A C T G T A A A T G C C C A C G A T A A A G T C C G T T T T...
          6430              6440              6450...
                                ... ALA  ASN  GLY  LEU  ASN  THR  LYS  VAL  SER  ALA
                                ... G C T A A T G G T T T A A A T A C C A A A G T G A G C G C G
                                ...           6460              6470              6480

 ALA  THR  VAL  GLU  SER  THR  ASP  ALA  ASN  GLY ...
G C A A C G G T G G A A A G C A C T G A T G C A A A C G G C...
          6490              6500              6510...
```

FIG.24F'

... ASP LYS VAL THR THR THR PHE VAL LYS THR
... G A T A A A G T G A C C A C A A C C T T T G T G A A A A C C
... 6520 6530 6540

ASP VAL GLU LEU PRO LEU THR GLN ILE TYR ...
G A T G T G G A A T T G C C T T T A A C G C A A A T C T A C...
6550 6560 6570...
... ASN THR ASP ALA ASN GLY ASN LYS ILE VAL
... A A T A C C G A T G C A A A C G G T A A T A A G A T C G T T
... 6580 6590 6600

LYS LYS ALA ASP GLY LYS TRP TYR GLU LEU ...
A A A A A A G C T G A C G G A A A A T G G T A T G A A C T G...
6610 6620 6630...
... ASN ALA ASP GLY THR ALA SER ASN LYS GLU
... A A T G C T G A T G G T A C G G C G A G T A A C A A A G A A
... 6640 6650 6660

VAL THR LEU GLY ASN VAL ASP ALA ASN GLY ...
G T G A C A C T T G G T A A C G T G G A T G C A A A C G G T...
6670 6680 6690...
... LYS LYS VAL VAL LYS VAL THR GLU ASN GLY
... A A G A A A G T T G T G A A A G T A A C C G A A A A T G G T
... 6700 6710 6720

FIG.24G'

```
 ALA  ASP  LYS  TRP  TYR  TYR  THR  ASN  ALA  ASP ...
G C G G A T A A G T G G T A T T A C A C C A A T G C T G A C...
          6730              6740              6750...
                ... GLY  ALA  ALA  ASP  LYS  THR  LYS  GLY  GLU  VAL
                ... G G T G C T G C G G A T A A A A C C A A A G G C G A A G T G
                ...           6760              6770              6780

 SER  ASN  ASP  LYS  VAL  SER  THR  ASP  GLU  LYS ...
A G C A A T G A T A A A G T T T C T A C C G A T G A A A A A...
          6790              6800              6810...
                ... HIS  VAL  VAL  ARG  LEU  ASP  PRO  ASN  ASN  GLN
                ... C A C G T T G T C C G C C T T G A T C C G A A C A A T C A A
                ...           6820              6830              6840

 SER  ASN  GLY  LYS  GLY  VAL  VAL  ILE  ASP  ASN ...
T C G A A C G G C A A A G G C G T G G T C A T T G A C A A T...
          6850              6860              6870...
                ... VAL  ALA  ASN  GLY  GLU  ILE  SER  ALA  THR  SER
                ... G T G G C T A A T G G C G A A A T T T C T G C C A C T T C C
                ...           6880              6890              6900

 THR  ASP  ALA  ILE  ASN  GLY  SER  GLN  LEU  TYR ...
A C C G A T G C G A T T A A C G G A A G T C A G T T G T A T...
          6910              6920              6930...
```

FIG.24H'

```
                                ... ALA VAL ALA LYS GLY VAL THR ASN LEU ALA
                                ... G C C G T G G C A A A A G G G G T A A C A A A C C T T G C T
                                ...              6940              6950              6960

 GLY GLN VAL ASN ASN LEU GLU GLY LYS VAL ...
G G A C A A G T G A A T A A T C T T G A G G G C A A A G T G...
             6970              6980              6990...
                                ... ASN LYS VAL GLY LYS ARG ALA ASP ALA GLY
                                ... A A T A A A G T G G G C A A A C G T G C A G A T G C A G G T
                                ...              7000              7010              7020

 THR ALA SER ALA LEU ALA ALA SER GLN LEU ...
A C A G C A A G T G C A T T A G C G G C T T C A C A G T T A...
             7030              7040              7050...
                                ... PRO GLN ALA THR MET PRO GLY LYS SER MET
                                ... C C A C A A G C C A C T A T G C C A G G T A A A T C A A T G
                                ...              7060              7070              7080

 VAL ALA ILE ALA GLY SER SER TYR GLN GLY ...
G T T G C T A T T G C G G G A A G T A G T T A T C A A G G T...
             7090              7100              7110...
                                ... GLN ASN GLY LEU ALA ILE GLY VAL SER ARG
                                ... C A A A A T G G T T T A G C T A T C G G G G T A T C A A G A
                                ...              7120              7130              7140
```

FIG.24I'

```
  ILE  SER  ASP  ASN  GLY  LYS  VAL  ILE  ILE  ARG ...
A T T T C C G A T A A T G G C A A A G T G A T T A T T C G C...
          7150                 7160                 7170....

...  LEU  SER  GLY  THR  THR  ASN  SER  GLN  GLY  LYS
... T T G T C A G G C A C A A C C A A T A G T C A A A G G T A A A
                     7180                 7190                 7200

...

  THR  GLY  VAL  ALA  ALA  GLY  VAL  GLY  TYR  GLN ...
A C A G G C G T T G C A G C A G G T G T T G G T T A C C A G...
          7210                 7220                 7230....

...  TRP  ***
... T G G T A A T A G A A T T C C G G G A T C C G C
                     7240                 7250

...
```

FIG.25A

NTHi strain 12 *hia* locus

```
          TYR  TYR  HIS  TRP  ***  PRO  THR  PRO ...
G A A T T C T A T T A C C A C T G G T A A C C A A C A C C T ...
              10                   20                   30 ...
                                ... ALA  ALA  THR  PRO  GLU  THR  ALA  GLN  GLN  ILE
                                ...G C T G C A A C G C C A G A A A C A G C A C A A C A A A T T
                                ...              40                   50                   60

 HIS  TRP  LEU  HIS  GLN  PHE  THR  LYS  ALA  ARG ...
C A C T G G C T A C A T C A A T T T A C C A A A G C T C G C ...
              70                   80                   90 ...
                                ... ILE  GLN  TRP  ARG  LYS  THR  HIS  SER  LEU  PHE
                                ...A T T C A A T G G C G C A A A A C C C A T T C C T T A T T C
                                ...              100                  110                  120

 PHE  LYS  GLU  LYS  PRO  ASP  TYR  ALA  PHE  VAL ...
T T T A A A G A A A A A C C C G A T T A T G C C T T T G T G ...
              130                  140                  150 ...
                                ... LEU  ALA  GLU  ASN  GLY  LYS  VAL  GLN  GLU  ILE
                                ...C T G G C A G A A A A C G G C A A A G T G C A A G A A A T C
                                ...              160                  170                  180

 LYS  ALA  GLU  TYR  ARG  ARG  ILE  ALA  ASN  GLN ...
A A A G C A G A A T A T C G C C G C A T T G C C A A T C A A ...
              190                  200                  210 ...
```

FIG.25B

```
                                    ... ILE  VAL  GLU  GLU  ALA  MET  ILE  ILE  ALA  ASN
                                    ...A T T G T G G A A G A A G C A A T G A T T A T T G C C A A C
                                    ...          220                  230                  240

 ILE  CYS  ALA  ALA  GLN  PHE  LEU  HIS  GLU  GLN ...
A T C T G C G C C G C C C A A T T T T T A C A C G A A C A G ...
             250                  260                  270 ...
                                    ... ALA  LYS  THR  GLY  ILE  PHE  ASN  ALA  HIS  SER
                                    ...G C A A A A A C A G G C A T T T T C A A C G C C C A C A G C
                                    ...          280                  290                  300

 GLY  PHE  ASP  LYS  LYS  TYR  LEU  GLU  ASN  ALA ...
                                                _____...
G G T T T T G A T A A A A A A T A C T T A G A A A A T G C G ...
             310                  320                  330 ...
                                    ... HIS  HIS  PHE  LEU  MET  ALA  ASN  LEU  ALA  ASN
                                    ..._____6431.SL (_____
                                    ...C A C C A T T T C T T A A T G G C A A A T T T A G C C A A T
                                    ...          340                  350                  360

 GLU  GLN  ASN  GLN  THR  GLU  LEU  ALA  GLU  ARG ...
G A A C A A A A T C A A A C T G A A C T G G C A G A A C G T ...
             370                  380                  390 ...
                                    ... TYR  SER  VAL  GLU  ASN  LEU  ALA  THR  LEU  ASN
                                    ...T A T T C A G T A G A A A A C T T A G C A A C C T T A A A C
                                    ...          400                  410                  420
```

FIG.25C

```
 GLY   TYR   CYS   GLN   MET   ARG   HIS   ASP   ILE   GLU  ...
G G C T A T T G C C A A A T G C G T C A C G A T A T T G A A ...
              430                   440                   450 ...
                                                  ... PRO   ILE   GLU   SER   ASP   TYR   LEU   GLU   LEU   ARG
                                                  ...C C C A T C G A A A G C G A T T A T T T A G A A C T G C G T
                                                  ...                   460                   470                   480

 LEU   ARG   ARG   TYR   LEU   THR   PHE   ALA   GLU   PHE  ...
T T A C G C C G T T A T T T A A C T T T C G C C G A A T T T ...
              490                   500                   510 ...
                                                  ... LYS   SER   GLU   LEU   ALA   PRO   HIS   PHE   GLY   LEU
                                                  ...A A A T C A G A A T T A G C A C C G C A C T T T G G T C T T
                                                  ...                   520                   530                   540

 GLY   LEU   GLU   GLY   TYR   ALA   THR   TRP   THR   SER  ...
G G T T T A G A A G G C T A T G C C A C T T G G A C A T C G ...
              550                   560                   570 ...
                                                  ... PRO   ILE   ARG   LYS   TYR   SER   ASP   MET   VAL   ASN
                                                  ...C C C A T C C G C A A A T A T T C A G A T A T G G T T A A T
                                                  ...                   580                   590                   600

 HIS   ARG   LEU   ILE   LYS   ALA   VAL   LEU   ALA   LYS  ...
                                                            ...
C A T C G C T T A A T C A A A G C C G T G C T G G C A A A A ...
              610                   620                   630 ...
```

FIG.25D

```
                                    ... GLN  PRO  TYR  GLU  LYS  PRO  GLN  ASN  ASP  VAL
                                    ...                                          _____
                                    ...C A G C C T T A T G A A A A A C C A C A A A A T G A C G T G
                                    ...          640                 650                 660

 LEU  ALA  ARG  LEU  GLN  GLU  SER  ARG  ARG  GLN ...
_____6432.SL (_____________________________________...
T T G G C A C G T T T G C A A G A G T C T C G C C G C C A A ...
             670                 680                 690 ...
                                    ... ASN  ARG  LEU  VAL  GLU  ARG  ASP  ILE  ALA  ASP
                                    ...  _____________
                                    ...A A T C G C C T A G T G G A A C G T G A T A T T G C C G A T
                                    ...          700                 710                 720

 TRP  LEU  TYR  CYS  ARG  TYR  LEU  ALA  ASP  LYS ...
T G G C T A T A T T G C C G T T A T C T T G C T G A C A A A ...
             730                 740                 750 ...
                                    ... VAL  ALA  GLU  ASN  VAL  GLU  PHE  ASN  ALA  GLU
                                    ...G T G G C T G A A A A T G T G G A A T T T A A T G C A G A A
                                    ...          760                 770                 780

 VAL  GLN  ASP  VAL  MET  ARG  ALA  GLY  LEU  ARG ...
G T G C A A G A T G T A A T G C G T G C A G G C T T A C G C ...
             790                 800                 810 ...
```

FIG.25E

```
                                    ... VAL  GLN  LEU  LEU  GLU  ASN  GLY  ALA  SER  LEU
                                    ...G T A C A A C T G C T C G A A A A T G G T G C A T C G C T A
                                    ...              820                  830                  840

 PHE  ILE  PRO  ALA  ALA  THR  LEU  HIS  ASN  ASN ...
T T T A T T C C T G C C G C C A C G T T G C A C A A C A A C ...
                850                  860                  870 ...
                                    ... LYS  GLU  GLU  ILE  GLN  LEU  ASN  PRO  ASP  GLU
                                    ...A A A G A A G A A A T A C A G C T A A A C C C T G A C G A A
                                    ...              880                  890                  900

 LEU  ALA  LEU  TYR  ILE  LYS  GLY  GLU  ARG  THR ...
C T C G C C C T C T A T A T A A A A G G C G A A C G C A C T ...
                910                  920                  930 ...
                                    ... TYR  LYS  ILE  GLY  ASP  ILE  VAL  LYS  VAL  LYS
                                    ...T A C A A A A T A G G C G A C A T T G T G A A A G T G A A A
                                    ...              940                  950                  960

 LEU  THR  GLU  VAL  LYS  GLU  ALA  THR  ARG  SER ...
C T C A C A G A A G T G A A A G A A G C A A C T C G C A G T ...
                970                  980                  990 ...
                                    ... ILE  VAL  GLY  GLU  ILE  LEU  GLN  ***      LEU  PRO
                                    ...A T T G T G G G C G A A A T A C T T C A A T A A A T T G C C
                                    ...              1000                 1010                 1020
```

FIG.25F

```
   PHE  GLN  TYR  VAL  THR  GLU  ASP  GLY  LYS  THR...
G T T C C A A T A T G T T A C G G A A G A C G G C A A A A C ...
            1030              1040              1050 ...
                              ...  VAL  VAL  LYS  VAL  GLY  ASN  GLU  TYR  TYR  GLU
                              ...C G T T G T G A A A G T G G G C A A T G A G T A T T A C G A
                              ...            1060              1070              1080

   ALA  LYS  GLN  ASP  GLY  SER  ALA  ASP  MET  ASP...
A G C C A A G C A A G A C G G T T C G G C G G A T A T G G A ...
                 (  6295.SL
            1090              1100              1110 ...
                              ...  LYS  LYS  VAL  LYS  ASN  GLY  GLU  LEU  VAL  LYS
                              ...T A A A A A A G T C A A A A A T G G C G A G C T G G T G A A
                              ...
                              ...            1120              1130              1140

   THR  LYS  VAL  LYS  LEU  VAL  SER  ALA  ASN  GLY...
A A C T A A A G T G A A A T T G G T A T C G G C A A A C G G ...
            1150              1160              1170 ...
                              ...  THR  ASN  PRO  VAL  LYS  ILE  SER  ASN  VAL  ALA
                              ...T A C A A A T C C G G T G A A A A T C A G C A A T G T T G C
                              ...            1180              1190              1200

   GLU  GLY  THR  GLU  ASP  THR  ASP  ALA  VAL  SER...
G G A A G G C A C G G A A G A T A C C G A T G C G G T C A G ...
            1210              1220              1230 ...
```

FIG.25G

```
                                    ...  PHE  LYS  GLN  LEU  LYS  ALA  LEU  GLN  ASN  LYS
                                    ...C T T T A A G C A G T T G A A A G C C T T G C A A A A C A A
                                    ...            1240              1250              1260

  GLN  VAL  THR  LEU  SER  ALA  SER  ASN  ALA  TYR...
A C A G G T T A C G T T A A G C G C G A G C A A T G C T T A ...
            1270              1280              1290 ...
                                    ...  ALA  ASN  GLY  GLY  SER  ASP  ALA  ASP  VAL  GLY
                                    ...T G C C A A T G G C G G T A G C G A T G C C G A C G T C G G
                                    ...            1300              1310              1320

  LYS  VAL  THR  GLN  THR  LEU  SER  ASN  GLY  LEU...
C A A G G T A A C T C A A A C T T T A A G C A A T G G T T T ...
            1330              1340              1350 ...
                                    ...  ASN  PHE  LYS  PHE  LYS  SER  THR  ASP  GLY  GLU
                                    ...G A A T T T T A A A T T T A A A T C C A C A G A C G G C G A
                                    ...            1360              1370              1380

  LEU  LEU  ASN  ILE  LYS  ALA  ASP  LYS  ASP  THR...
G T T G T T G A A C A T C A A A G C A G A C A A G G A C A C ...
            1390              1400              1410 ...
                                    ...  VAL  THR  ILE  THR  ARG  ALA  SER  GLY  ALA  ASN
                                    ...G G T T A C C A T T A C G C G G G C A A G C G G T G C G A A
                                    ...            1420              1430              1440
```

FIG.25H

```
   GLY  ALA  ALA  ALA  THR  ASP  ALA  ASP  LYS  ILE...
TGGTGCGGCGGCGACTGATGCCGACAAGAT...
          1450          1460          1470 ...
                                ...  LYS  VAL  ALA  SER  ASP  GLY  ILE  SER  ALA  GLY
                                ...TAAAGTGGCTTCAGACGGCATTAGCGCGGG
                                ...          1480          1490          1500

   ASN  LYS  ALA  VAL  LYS  ASN  VAL  ALA  ALA  GLY...
TAATAAAGCAGTTAAAAACGTCGCGGCAGG...
                                                  ...
          1510          1520          1530 ...
                                ...  GLU  ILE  SER  ALA  THR  SER  THR  ASP  ALA  ILE
                                ...CGAAATTTCCGCCACTTCCACCGATGCGAT
                                ...                    ( 6271.SL
                                ...          1540          1550          1560

   ASN  GLY  SER  GLN  LEU  TYR  ALA  VAL  ALA  LYS...
TAACGGCAGTCAGTTGTATGCCGTGGCAAA...
          1570          1580          1590 ...
                                ...  GLY  VAL  THR  ASN  LEU  ALA  GLY  GLN  VAL  ASN
                                ...GGGGGTAACAAACCTTGCTGGACAAGTGAA
                                ...          1600          1610          1620

   LYS  VAL  GLY  LYS  ARG  ALA  ASP  ALA  GLY  THR...
TAAAGTGGGCAAACGTGCAGATGCAGGTAC...
          1630          1640          1650 ...
```

FIG.25I

```
                                    ...  ALA  SER  ALA  LEU  ALA  ALA  SER  GLN  LEU  PRO
                                    ...A G C A A G T G C A T T A G C G G C T T C A C A G T T A C C
                                    ...              1660              1670              1680

  GLN  ALA  SER  MET  PRO  GLY  LYS  SER  MET  VAL...
A C A A G C C T C T A T G C C G G G T A A A T C A A T G G T ...
          1690              1700              1710 ...
                                    ...  SER  ILE  ALA  GLY  SER  SER  TYR  GLN  GLY  GLN
                                    ...T T C T A T T G C G G G A A G T A G T T A T C A A G G T C A
                                    ...              1720              1730              1740

  SER  GLY  LEU  ALA  ILE  GLY  VAL  SER  ARG  ILE...
A A G T G G T T T A G C T A T C G G G G T A T C A A G A A T ...
          1750              1760              1770 ...
                                    ...  SER  ASP  ASN  GLY  LYS  LEU  ILE  ILE  ARG  LEU
                                    ...T T C C G A T A A T G G C A A A T T G A T T A T T C G C T T
                                    ...              1780              1790              1800

  SER  GLY  THR  THR  ASN  SER  GLN  GLY  LYS  THR...
G T C A G G C A C A A C C A A T A G C C A A G G T A A A A C ...
          1810              1820              1830 ...
                                    ...  GLY  VAL  ALA  ALA  GLY  VAL  GLY  TYR  GLN  TRP
                                    ...A G G C G T T G C A G C A G G T G T T G G T T A C C A G T G
                                    ...              1840              1850              1860

   ***  ***
G T A A T A G A A T T C
          1870
```

FIG.26A

```
ATG AAC AAA ATT TTT AAC GTT ATT TGG AAT GTT GTG ACT CAA ACT TGG      48
Met Asn Lys Ile Phe Asn Val Ile Trp Asn Val Val Thr Gln Thr Trp
        2130                2135                2140

GTT GTC GTA TCT GAA CTC ACT CGC ACC CAC ACC AAA TGC GCC TCC GCC      96
Val Val Val Ser Glu Leu Thr Arg Thr His Thr Lys Cys Ala Ser Ala
    2145                2150                2155

ACC GTG GCG GTT GCC GTA TTG GCA ACC CTG TTG TCC GCA ACG GTT GAG     144
Thr Val Ala Val Ala Val Leu Ala Thr Leu Leu Ser Ala Thr Val Glu
2160                2165                2170                2175

GCG AAC AAC AAT ACT CCT GTT ACG AAT AAG TTG AAG GCT TAT GGC GAT     192
Ala Asn Asn Asn Thr Pro Val Thr Asn Lys Leu Lys Ala Tyr Gly Asp
                2180                2185                2190

GCG AAT TTT AAT TTC ACT AAT AAT TCG ATA GCA GAT GCA GAA AAA CAA     240
Ala Asn Phe Asn Phe Thr Asn Asn Ser Ile Ala Asp Ala Glu Lys Gln
            2195                2200                2205

GTT CAA GAG GCT TAT AAA GGT TTA TTA AAT CTA AAT GAA AAA AAT GCG     288
Val Gln Glu Ala Tyr Lys Gly Leu Leu Asn Leu Asn Glu Lys Asn Ala
        2210                2215                2220
```

FIG.26B

```
AGT GAT AAA CTG TTG GTG GAG GAC AAT ACT GCG GCG ACC GTA GGC AAT      336
Ser Asp Lys Leu Leu Val Glu Asp Asn Thr Ala Ala Thr Val Gly Asn
    2225                2230                2235

TTG CGT AAA TTG GGC TGG GTA TTG TCT AGC AAA AAC GGC ACA AGG AAC      384
Leu Arg Lys Leu Gly Trp Val Leu Ser Ser Lys Asn Gly Thr Arg Asn
2240                2245                2250                2255

GAG AAA AGC CAA CAA GTC AAA CAT GCG GAT GAA GTG TTG TTT GAA GGC      432
Glu Lys Ser Gln Gln Val Lys His Ala Asp Glu Val Leu Phe Glu Gly
                2260                2265                2270

AAA GGC GGT GTG CAG GTT ACT TCC ACC TCT GAA AAC GGC AAA CAC ACC      480
Lys Gly Gly Val Gln Val Thr Ser Thr Ser Glu Asn Gly Lys His Thr
            2275                2280                2285

ATT ACC TTT GCT TTA GCG AAA GAC CTT GGT GTG AAA ACT GCG ACT GTG      528
Ile Thr Phe Ala Leu Ala Lys Asp Leu Gly Val Lys Thr Ala Thr Val
        2290                2295                2300

AGT GAT ACC TTA ACG ATT GGC GGT GGT GCT GCT GCA GGT GCT ACA ACA      576
Ser Asp Thr Leu Thr Ile Gly Gly Gly Ala Ala Ala Gly Ala Thr Thr
    2305                2310                2315
```

FIG.26C

```
ACA CCG AAA GTG AAT GTA ACT AGT ACA ACT GAT GGC TTG AAG TTC GCT      624
Thr Pro Lys Val Asn Val Thr Ser Thr Thr Asp Gly Leu Lys Phe Ala
2320                2325                2330                2335

AAA GAT GCT GCG GGT GCT AAT GGC GAT ACT ACG GTT CAC TTG AAT GGT      672
Lys Asp Ala Ala Gly Ala Asn Gly Asp Thr Thr Val His Leu Asn Gly
                    2340                2345                2350

ATT GGT TCA ACC TTG ACA GAC ACG CTT GTG GGT TCT CCT GCT ACT CAT      720
Ile Gly Ser Thr Leu Thr Asp Thr Leu Val Gly Ser Pro Ala Thr His
            2355                2360                2365

ATT GAC GGA GGA GAT CAA AGT ACG CAT TAC ACT CGT GCA GCA AGT ATC      768
Ile Asp Gly Gly Asp Gln Ser Thr His Tyr Thr Arg Ala Ala Ser Ile
        2370                2375                2380

AAG GAT GTC TTG AAT GCG GGT TGG AAT ATC AAG GGT GTT AAA GCT GGC      816
Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys Gly Val Lys Ala Gly
    2385                2390                2395

TCA ACA ACT GGT CAA TCA GAA AAT GTC GAT TTT GTT CAT ACT TAC GAT      864
Ser Thr Thr Gly Gln Ser Glu Asn Val Asp Phe Val His Thr Tyr Asp
2400                2405                2410                2415
```

FIG.26D

```
ACT GTT GAG TTC TTG AGT GCG GAT ACA GAG ACC ACG ACT GTT ACT GTA      912
Thr Val Glu Phe Leu Ser Ala Asp Thr Glu Thr Thr Thr Val Thr Val
                2420                2425                2430

GAT AGC AAA GAA AAC GGT AAG AGA ACC GAA GTT AAA ATC GGT GCG AAG      960
Asp Ser Lys Glu Asn Gly Lys Arg Thr Glu Val Lys Ile Gly Ala Lys
            2435                2440                2445

ACT TCT GTT ATC AAA GAA AAA GAC GGT AAG TTA TTT ACT GGA AAA GCT     1008
Thr Ser Val Ile Lys Glu Lys Asp Gly Lys Leu Phe Thr Gly Lys Ala
        2450                2455                2460

AAC AAA GAG ACA AAT AAA GTT GAT GGT GCT AAC GCG ACT GAA GAT GCA     1056
Asn Lys Glu Thr Asn Lys Val Asp Gly Ala Asn Ala Thr Glu Asp Ala
    2465                2470                2475

GAC GAA GGC AAA GGC TTA GTG ACT GCG AAA GAT GTG ATT GAC GCA GTG     1104
Asp Glu Gly Lys Gly Leu Val Thr Ala Lys Asp Val Ile Asp Ala Val
2480                2485                2490                2495

AAT AAG ACT GGT TGG AGA ATT AAA ACA ACC GAT GCT AAT GGT CAA AAT     1152
Asn Lys Thr Gly Trp Arg Ile Lys Thr Thr Asp Ala Asn Gly Gln Asn
                2500                2505                2510
```

FIG.26E

```
GGC GAC TTC GCA ACT GTT GCA TCA GGC ACA AAT GTA ACC TTT GCT AGT      1200
Gly Asp Phe Ala Thr Val Ala Ser Gly Thr Asn Val Thr Phe Ala Ser
            2515                2520                2525

GGT AAT GGT ACA ACT GCG ACT GTA ACT AAT GGC ACC GAT GGT ATT ACC      1248
Gly Asn Gly Thr Thr Ala Thr Val Thr Asn Gly Thr Asp Gly Ile Thr
        2530                2535                2540

GTT AAG TAT GAT GCG AAA GTT GGC GAC GGC TTA AAA CTA GAT GGC GAT      1296
Val Lys Tyr Asp Ala Lys Val Gly Asp Gly Leu Lys Leu Asp Gly Asp
    2545                2550                2555

AAA ATC GCT GCA GAT ACG ACC GCA CTT ACT GTG AAT GAT GGT AAG AAC      1344
Lys Ile Ala Ala Asp Thr Thr Ala Leu Thr Val Asn Asp Gly Lys Asn
2560                2565                2570                2575

GCT AAT AAT CCG AAA GGT AAA GTG GCT GAT GTT GCT TCA ACT GAC GAG      1392
Ala Asn Asn Pro Lys Gly Lys Val Ala Asp Val Ala Ser Thr Asp Glu
                2580                2585                2590

AAG AAA TTG GTT ACA GCA AAA GGT TTA GTA ACA GCC TTA AAC AGT CTA      1440
Lys Lys Leu Val Thr Ala Lys Gly Leu Val Thr Ala Leu Asn Ser Leu
            2595                2600                2605
```

FIG.26F

```
AGC TGG ACT ACA ACT GCT GCT GAG GCG GAC GGT GGT ACG CTT GAT GGA      1488
Ser Trp Thr Thr Thr Ala Ala Glu Ala Asp Gly Gly Thr Leu Asp Gly
        2610                2615                2620

AAT GCA AGT GAG CAA GAA GTT AAA GCG GGC GAT AAA GTA ACC TTT AAA      1536
Asn Ala Ser Glu Gln Glu Val Lys Ala Gly Asp Lys Val Thr Phe Lys
    2625                2630                2635

GCA GGC AAG AAC TTA AAA GTG AAA CAA GAG GGT GCG AAC TTT ACT TAT      1584
Ala Gly Lys Asn Leu Lys Val Lys Gln Glu Gly Ala Asn Phe Thr Tyr
2640                2645                2650                2655

TCA CTG CAA GAT GCT TTA ACA GGC TTA ACG AGC ATT ACT TTA GGT ACA      1632
Ser Leu Gln Asp Ala Leu Thr Gly Leu Thr Ser Ile Thr Leu Gly Thr
                2660                2665                2670

GGA AAT AAT GGT GCG AAA ACT GAA ATC AAC AAA GAC GGC TTA ACC ATC      1680
Gly Asn Asn Gly Ala Lys Thr Glu Ile Asn Lys Asp Gly Leu Thr Ile
            2675                2680                2685

ACA CCA GCA AAT GGT GCG GGT GCA AAT AAT GCA AAC ACC ATC AGC GTA      1728
Thr Pro Ala Asn Gly Ala Gly Ala Asn Asn Ala Asn Thr Ile Ser Val
        2690                2695                2700
```

FIG.26G

```
ACC AAA GAC GGC ATT AGT GCG GGC GGT CAG TCG GTT AAA AAC GTT GTG      1776
Thr Lys Asp Gly Ile Ser Ala Gly Gly Gln Ser Val Lys Asn Val Val
    2705                2710                2715

AGC GGA CTG AAG AAA TTT GGT GAT GCG AAT TTC GAT CCG CTG ACT AGC      1824
Ser Gly Leu Lys Lys Phe Gly Asp Ala Asn Phe Asp Pro Leu Thr Ser
2720                2725                2730                2735

TCC GCC GAC AAC TTA ACG AAA CAA AAT GAC GAT GCC TAT AAA GGC TTG      1872
Ser Ala Asp Asn Leu Thr Lys Gln Asn Asp Asp Ala Tyr Lys Gly Leu
                2740                2745                2750

ACC AAT TTG GAT GAA AAA GGT ACA GAC AAG CAA ACT CCA GTT GTT GCC      1920
Thr Asn Leu Asp Glu Lys Gly Thr Asp Lys Gln Thr Pro Val Val Ala
            2755                2760                2765

GAC AAT ACC GCC GCA ACC GTG GGC GAT TTG CGC GGC TTG GGC TGG GTC      1968
Asp Asn Thr Ala Ala Thr Val Gly Asp Leu Arg Gly Leu Gly Trp Val
        2770                2775                2780

ATT TCT GCG GAC AAA ACC ACA GGC GGC TCA ACG GAA TAT CAC GAT CAA      2016
Ile Ser Ala Asp Lys Thr Thr Gly Gly Ser Thr Glu Tyr His Asp Gln
    2785                2790                2795
```

FIG.26H

```
GTT CGG AAT GCG AAC GAA GTG AAA TTC AAA AGC GGC AAC GGT ATC AAT      2064
Val Arg Asn Ala Asn Glu Val Lys Phe Lys Ser Gly Asn Gly Ile Asn
2800                2805                2810                2815

GTT TCC GGT AAA ACG GTC AAC GGT AGG CGT GAA ATT ACT TTT GAA TTG      2112
Val Ser Gly Lys Thr Val Asn Gly Arg Arg Glu Ile Thr Phe Glu Leu
                    2820                2825                2830

GCT AAA GGT GAA GTG GTT AAA TCG AAT GAA TTT ACC GTC AAA GAA ACC      2160
Ala Lys Gly Glu Val Val Lys Ser Asn Glu Phe Thr Val Lys Glu Thr
            2835                2840                2845

AAT GGA AAG GAA ACG AGC CTG GTT AAA GTT GGC GAT AAA TAT TAC AGC      2208
Asn Gly Lys Glu Thr Ser Leu Val Lys Val Gly Asp Lys Tyr Tyr Ser
        2850                2855                2860

AAA GAG GAT ATT GAC TTA ACA ACA GGT CAG CCT AAA TTA AAA GAT GGC      2256
Lys Glu Asp Ile Asp Leu Thr Thr Gly Gln Pro Lys Leu Lys Asp Gly
    2865                2870                2875

AAT ACA GTT GCT GCG AAA TAT CAA GAT AAA GGT GGC AAA GTC GTT TCT      2304
Asn Thr Val Ala Ala Lys Tyr Gln Asp Lys Gly Gly Lys Val Val Ser
2880                2885                2890                2895
```

FIG.26I

```
GTA ACG GAT AAT ACT GAA GCT ACC ATA ACC AAC AAA GGT TCT GGC TAT      2352
Val Thr Asp Asn Thr Glu Ala Thr Ile Thr Asn Lys Gly Ser Gly Tyr
                2900                2905                2910

GTA ACA GGT AAC CAA GTG GCA GAT GCG ATT GCG AAA TCA GGC TTT GAG      2400
Val Thr Gly Asn Gln Val Ala Asp Ala Ile Ala Lys Ser Gly Phe Glu
            2915                2920                2925

CTT GGC TTG GCT GAT GAA GCT GAT GCG AAA CGG GCG TTT GAT GAT AAG      2448
Leu Gly Leu Ala Asp Glu Ala Asp Ala Lys Arg Ala Phe Asp Asp Lys
        2930                2935                2940

ACA AAA GCC TTA TCT GCT GGT ACA ACG GAA ATT GTA AAT GCC CAC GAT      2496
Thr Lys Ala Leu Ser Ala Gly Thr Thr Glu Ile Val Asn Ala His Asp
    2945                2950                2955

AAA GTC CGT TTT GCT AAT GGT TTA AAT ACC AAA GTG AGC GCG GCA ACG      2544
Lys Val Arg Phe Ala Asn Gly Leu Asn Thr Lys Val Ser Ala Ala Thr
2960                2965                2970                2975

GTG GAA AGC ACC GAT GCA AAC GGC GAT AAA GTG ACC ACA ACC TTT GTG      2592
Val Glu Ser Thr Asp Ala Asn Gly Asp Lys Val Thr Thr Thr Phe Val
                2980                2985                2990
```

FIG.26J

```
AAA ACC GAT GTG GAA TTG CCT TTA ACG CAA ATC TAC AAT ACC GAT GCA      2640
Lys Thr Asp Val Glu Leu Pro Leu Thr Gln Ile Tyr Asn Thr Asp Ala
            2995                3000                3005

AAC GGT AAG AAA ATC ACT AAA GTT GTC AAA GAT GGG CAA ACT AAA TGG      2688
Asn Gly Lys Lys Ile Thr Lys Val Val Lys Asp Gly Gln Thr Lys Trp
        3010                3015                3020

TAT GAA CTG AAT GCT GAC GGT ACG GCT GAT ATG ACC AAA GAA GTT ACC      2736
Tyr Glu Leu Asn Ala Asp Gly Thr Ala Asp Met Thr Lys Glu Val Thr
    3025                3030                3035

CTC GGT AAC GTG GAT TCA GAC GGC AAG AAA GTT GTG AAA GAC AAC GAT      2784
Leu Gly Asn Val Asp Ser Asp Gly Lys Lys Val Val Lys Asp Asn Asp
3040                3045                3050                3055

GGC AAG TGG TAT CAC GCC AAA GCT GAC GGT ACT GCG GAT AAA ACC AAA      2832
Gly Lys Trp Tyr His Ala Lys Ala Asp Gly Thr Ala Asp Lys Thr Lys
                3060                3065                3070

GGC GAA GTG AGC AAT GAT AAA GTT TCT ACC GAT GAA AAA CAC GTT GTC      2880
Gly Glu Val Ser Asn Asp Lys Val Ser Thr Asp Glu Lys His Val Val
            3075                3080                3085
```

FIG.26K

2928
AGC CTT GAT CCA AAT GAT CAA TCA AAA GGT AAA GGT GTC GTG ATT GAC
Ser Leu Asp Pro Asn Asp Gln Ser Lys Gly Lys Gly Val Val Ile Asp
3090 3095 3100

2976
AAT GTG GCT AAT GGC GAT ATT TCT GCC ACT TCC ACC GAT GCG ATT AAC
Asn Val Ala Asn Gly Asp Ile Ser Ala Thr Ser Thr Asp Ala Ile Asn
3105 3110 3115

3024
GGA AGT CAG TTG TAT GCT GTG GCA AAA GGG GTA ACA AAC CTT GCT GGA
Gly Ser Gln Leu Tyr Ala Val Ala Lys Gly Val Thr Asn Leu Ala Gly
3120 3125 3130 3135

3072
CAA GTG AAT AAT CTT GAG GGC AAA GTG AAT AAA GTG GGC AAA CGT GCA
Gln Val Asn Asn Leu Glu Gly Lys Val Asn Lys Val Gly Lys Arg Ala
3140 3145 3150

3120
GAT GCA GGT ACA GCA AGT GCA TTA GCG GCT TCA CAG TTA CCA CAA GCC
Asp Ala Gly Thr Ala Ser Ala Leu Ala Ala Ser Gln Leu Pro Gln Ala
3155 3160 3165

3168
ACT ATG CCA GGT AAA TCA ATG GTT GCT ATT GCG GGA AGT AGT TAT CAA
Thr Met Pro Gly Lys Ser Met Val Ala Ile Ala Gly Ser Ser Tyr Gln
3170 3175 3180

FIG.26L

```
GGT CAA AAT GGT TTA GCT ATC GGG GTA TCA AGA ATT TCC GAT AAT GGC      3216
Gly Gln Asn Gly Leu Ala Ile Gly Val Ser Arg Ile Ser Asp Asn Gly
    3185                3190                3195

AAA GTG ATT ATT CGC TTG TCA GGC ACA ACC AAT AGT CAA GGT AAA ACA      3264
Lys Val Ile Ile Arg Leu Ser Gly Thr Thr Asn Ser Gln Gly Lys Thr
3200                3205                3210                3215

GGC GTT GCA GCA GGT GTT GGT TAC CAG TGG                              3294
Gly Val Ala Ala Gly Val Gly Tyr Gln Trp
                3220                3225
```

FIG.27A

Alignment of NTHi strain 12 5′ ORF with HI1733 from H. influenzae strain Rd

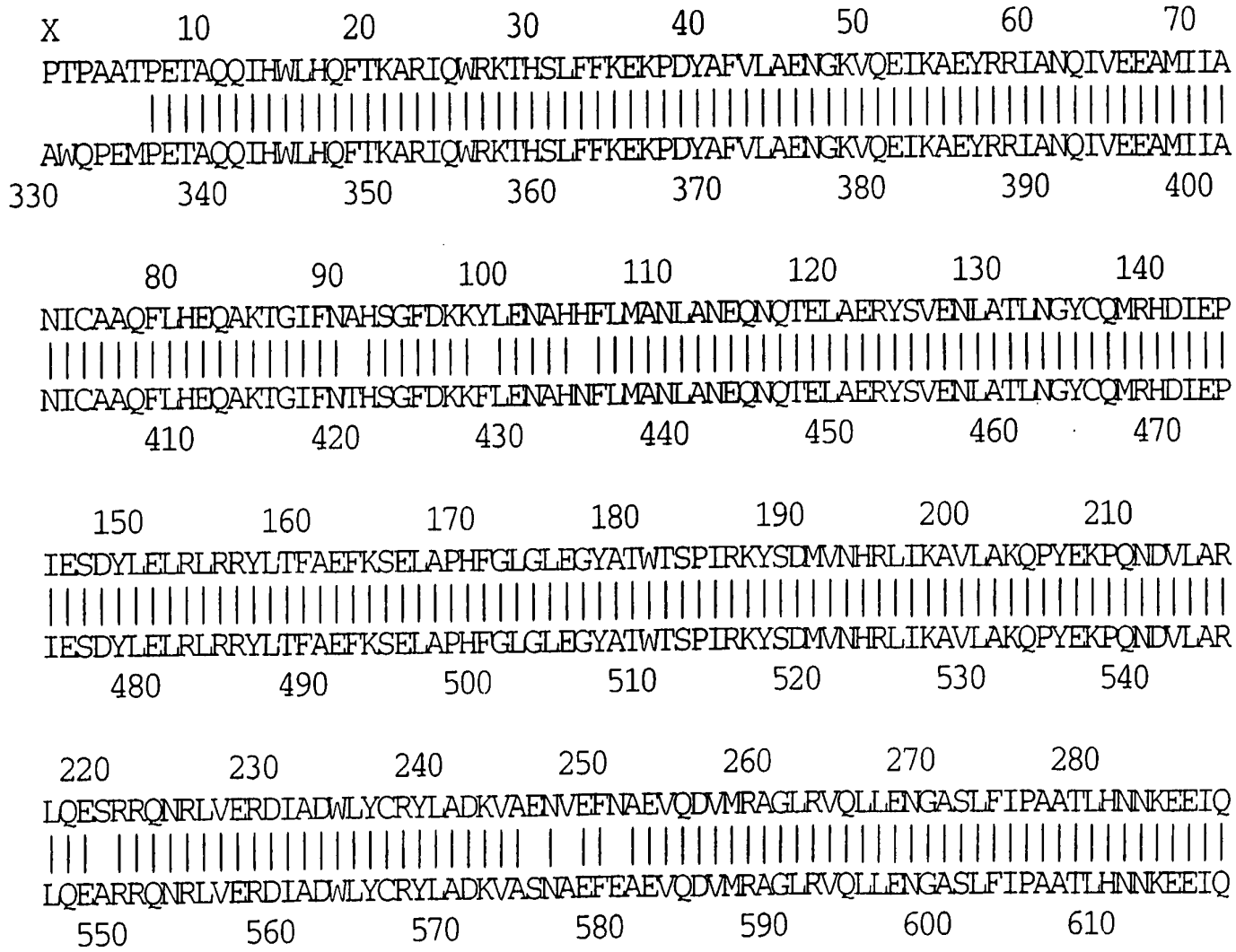


FIG.27B

```
290        300        310        320        330
 LNPDELALYIKGERTYKIGDIVKVKLTEVKEATRSIVGEILQ
 |||||||||||||||||||| ||||||||||||||||||||
 LNPDELALYIKGERTYKIGDMVKVKLTEVKEATRSIVGEILQ
 620        630        640        650        X
```

```
;       ##cross-references GB:L42023; TIGR:HI1733
;       ##note          named as homolog to a protein from Escherichia coli
; SUMMARY          #length 659  #molecular-weight 75782  #checksum 8365
```

A64139

MFQDNPLLAQLKQQIHDSKEQVEGVVKSTDKAYGFLECDKKTYFIAPPSMKKVMHGDKIKATIEKQGDKE
QAEPEALIEPMLTRFIAKVRFNKDKKLQVLVDHPSINQPIGAQQAKSVKEELQEGDWVVANLKTHPLRDD
RFFYATINQLICRADDELAPWWVTLARHEQSRYPVRGAEPYEMLDQKTRENLTALHFVTIDSESTMDMDD
ALYIEPIAQNSTQIGWKLVVAIADPTAYIALDSQIEQEAKQRCFTNYLPGFNIPMLPRELSDELCSLIAN
ETRPALVCYIETDLTGNITAKPHFVSAYVQSKAKLAYNKVSDYLEQADNAWQPEMPETAQQIHWLHQFTK
ARIQWRKTHSLFFKEKPDYAFVLAENGKVQEIKAEYRRIANQIVEEAMIIANICAAQFLHEQAKTGIFNT
HSGFDKKFLENAHNFLMANLANEQNQTELAERYSVENLATLNGYCQMRHDIEPIESDYLELRLRRYLTFA
EFKSELAPHFGLGLEGYATWTSPIRKYSDMVNHRLIKAVLAKQPYEKPQNDVLARLQEARRQNRLVERDI
ADWLYCRYLADKVASNAEFEAEVQDVMRAGLRVQLLENGASLFIPAATLHNNKEEIQLNPDELALYIKGE
RTYKIGDMVKVKLTEVKEATRSIVGEILQ

FIG.28A

Alignment of *H. influenzae* Hia/Hsf and *M. catarrhalis* 200 kDa proteins

```
        10         20         30         40         50           ...
MNKIFNVIWNVMIQIWAVVSELTRAHTKRASATVAAAVLATVLSATVQA-S----------...
..........V....V.......T...C......V.....L.......N----------...
..........V....V..........C......V.....A....AE.NN----------...
..........V....V..........C......V.....A....AE.NN----------...
.................................T.....L..T....TT----------...
..........V....V.......T...C......V.....L.....E.NN----------...
.................................T.....Q....AE.NS----------...
.....N.........V.......T.........ET.....L.F.....NATDEDEELDPVV...
...............V.......T.........ET.....L.F.....NATDEDEELDPVV...
.....K.....V....V............T.....T.............IN---------DA...
..H.YK..F.KA.G.FMA.A.YAKS.STGGGSCATGQ.GSVCTLSFARIAALAVLVIGATLS...
..H.YK..F.KA.G.FMA.A.CAKS.SGGSSSSTAGQ.GSSPVIRLTRVATLAILVIGATLN...
** ** ****  * **  *** *****  ***  *  * *                      ...

                                ...
                                ...----------------------------------------  33
                                ...----------------------------------------  32
                                ...----------------------------------------  29
                                ...----------------------------------------  K22
                                ...----------------------------------------  M4071
                                ...----------------------------------------  11
                                ...----------------------------------------  K9
                                ...RTAPVLSFHSDKEGTGEKEVTENSNWGIYFDNKGVLKA--------  HSF
```

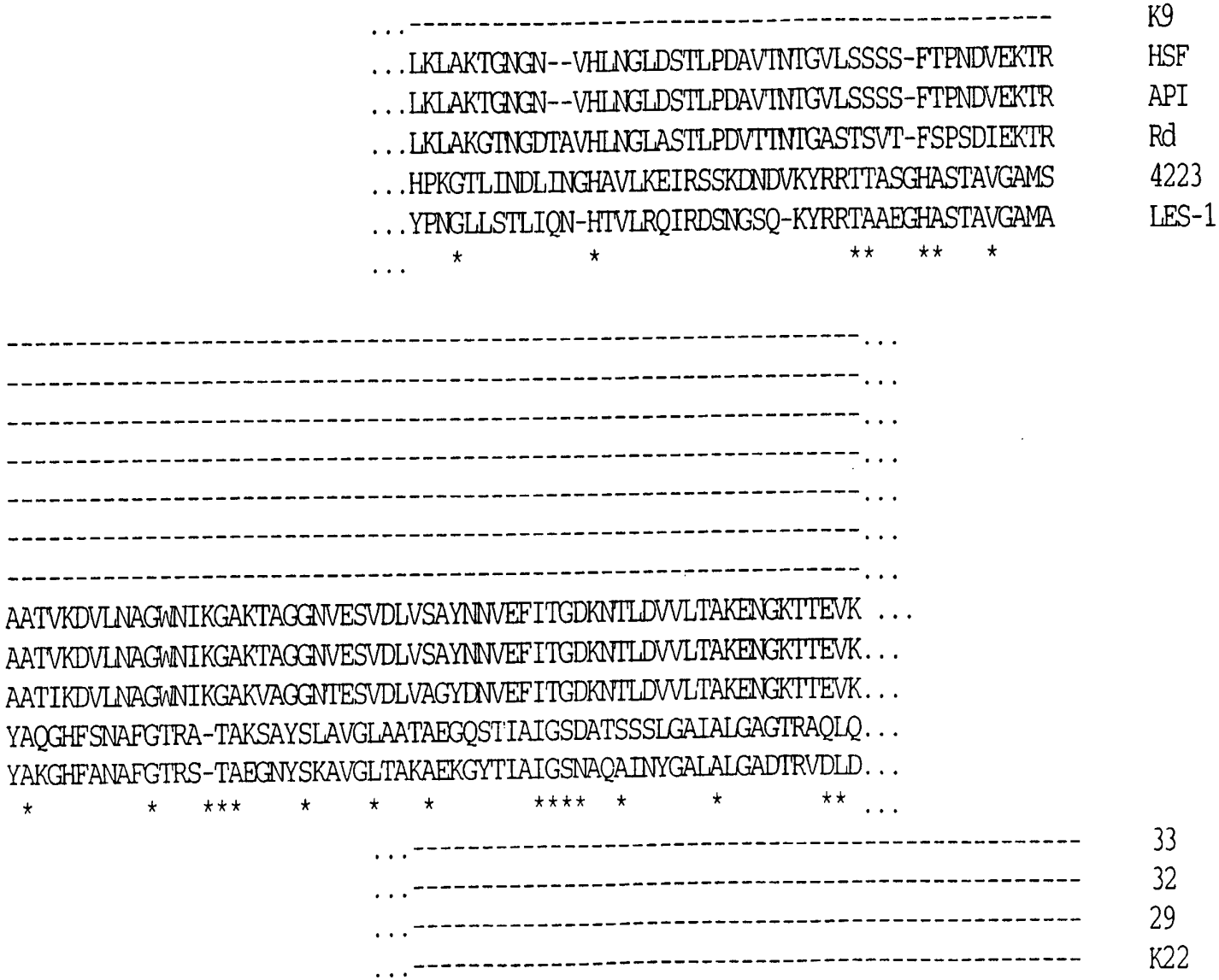
FIG.28B

```
...RTAPVLSFHSDKEGTGEKEVTENSNWGIYFHNKGVLKA--------        API
...GTFVKVQSTEDDIEDSAATKDDNKNQALKAGDTLTLKA--------        Rd
...GSAYAQKKDTKHIAIGEQNQPRRSGTAKADGDRAIAIGENANAQGG        4223
...GSAYAQN-NSK-AIFGTTGNNDN---ASASNEASIAIGSLAKAHAN        LES-1
...          *     *          *    *     *

----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
GAITLKAGDNLKIKQNTDESTNASSFTYSLKKDLTDLTSVATEKLSFGANGDKVDITSDANG...
GAITLKAGDNLKIKQ----STNASSFTYSLKKDLTDLTSVATEKLSFGANGDKVDITSDANG...
GKN-LKAKLDQGGKSVTFALAKDLDVKTAKVSDTLTIGGNTPAAGGATP---KVSITSTADG...
QAIAIGSSNKTVNG-SSLDKIGTDITGQESIAIGGDVKASGDASIAIGSDDLHLLDQHGNPK...
QAIAIGGSKPDPRNQAANQKAGSHAKGKESIAIGGDVLAEGDASIAIGSDDLYLDRNSTNSK...
   **          *                 * * * *  * * *   *          ...

                        ...----------------------------------------------        33
                        ...----------------------------------------------        32
                        ...----------------------------------------------        29
                        ...----------------------------------------------        K22
                        ...----------------------------------------------        M4071
                        ...----------------------------------------------        11
```

FIG.28C



SUBSTITUTE SHEET (RULE 26)

FIG.28D

```
...--------------------------------------------------   M4071
...--------------------------------------------------   11
...--------------------------------------------------   K9
...FTPKT---SVIKEKD----GKLFTGKENNDTNKV--TSNTA--------   HSF
...FTPKT---SVIKEKD----GKLFTGKENNDTNKV--TSNTA--------   API
...FTPKT---SVIKDNN----GKLLTGKQLKDANTG--TATNA--------   Rd
...GSIALGQGSVVTQSD-NNSRPAYTPNTQALDPKFQ-ATNNTKAGPLSIG   4223
...YGIALGYGSQILNNNNNNNNKAYVPEGNGSNIKSSKATGNGLF---SIG   LES-1
...  *      * * * *                    **   *

-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
TDNTDEGNGLVTAKAVI-DAVNKAGWTVKTTTANGQNGDFATVASGTNVTFESGDGTTASVT...
TDNTDEGNGLVTAKAVI-DAVNKAGWTVKTTTANGQNGDFATVASGTNVTFESGDGTTASVT...
TEDTDEAMA*                                                    ...
 *RYRRGNGLVTAKTVI-EAVNKSGWRVKTTTANGQNDDFATVASGTNVTFANGNGTTASVT...
SNSIKRKIINVGAGVNKTDAVNVAQLEAVVKWAKERRITFQGDDNSTDVKIGLDNTLTIKGG...
SSTIKRKIINVGAGYEDTDAVNVAQLKAVENLAK-RQITFKGDDNGTGVKKKLGETLTIKGG...
*         ***     **** *        *      * *   ** *     ** *   *...

                         ...-----------------------------------------   33
```

FIG.28E

```
32
29
K22
M4071
11
K9
HSF
API
Rd
Rd
4223
LES-1

...------------------------------------------------------------
...------------------------------------------------------------
...------------------------------------------------------------
...------------------------------------------------------------
...------------------------------------------------------------
...------------------------------------------------------------
...------------------------------------------------------------
...KDTNGNGITVKYD-ALVGDGLKFDSDKKIVADTTALTVTG----
...KDTNGNGITVKYD-ALVGDGLKFDSDKKIVADTTALTVTG---
...
...NSTD--GITVKYE-ALVGDGLKIDGDQKIVADTTALTVTG----
...AETNA--LTDNN- IGVVKEADNSGLKVKLAKTLNNLTEVTTTL
...-ETQADKLTDNNNIGVVTD-NNTGLKVKLAKNLSGLETVSTKNL
   **** **  * * * **     **     *
...
------------------------------------------------------------...
------------------------------------------------------------...
------------------------------------------------------------...
------------------------------------------------------------...
------------------------------------------------------------...
------------------------------------------------------------...
------------------------------------------------------------...
GKVAEIAKEDDKKLVNAGDLVTALGNLSWKAKAEADTD--GALEGISKDQEVKAGETVTFK...
GKVAEIAKEDDKKLVNAGDLVTALGNLSWKAKAEADTDTDGALEGISKDQEVKAGETVTFK...
GKVAEIAKEDDKKLVNAGDLVTALGNLSWKAKAEADTD--GALEGTSKDQEVKAGETVTFK...
GKVAEIAKEDDKKLVNAGDLVTALGNLSWKAKAEADTD--GALEGTSKDQEVKAGETVTFK...
NATTTVKVGSSSSTTAELLSDSLTFTQPNTGSQSTSKTVYGVNGVKFTNNAETTAAIGTT-R...
```

FIG.28F

```
TASEKVTVGSGNN-TAELQSGGLTFT-PTTNA-STDKTVYGTDGLKFTDNSN-TALEDTT-R...
      *       *     * **   * *  *  *     * * *  *   * *...
                        ...-------------------------------------  33
                        ...-------------------------------------  32
                        ...-------------------------------------  29
                        ...-------------------------------------  K22
                        ...-------------------------------------  M4071
                        ...-------------------------------------  11
                        ...-------------------------------------  K9
                        ...AGKNLKVKQDGANFTYSLQDALTGLTSATLGGTTNGGNDA  HSF
                        ...AGKNLKVKQDGANFTYSLQDALTGLTSATLGGTTNGGNDA  API
                        ...AGKNLKVKQDGANFTYSLQDALTGLTSATLGGTTNGGNDA  Rd
                        ...ITRDKIGFARD-GDVDE----------------------  4223
                        ...ITKDKIGFSNKAGTVDENKPYLDKDKLKVGNSTLNNGGLT  LES-1
                        ... ***      *        *        ** * *  *
                                                60        70        ...
 ------------------------------AGSTTGTNSLNVYGK-NNSNFNSANNSIA...
------------------------------------------------------------...
------------------------------NN--.SV..G..A..D---T...TT........
------------------------------N--.SV..G..A..D---T...TT........
------------------------------T.G..S..G.KA..ST..P...A.G..AT...
------------------------------N--.PV..K.KA..D-A.F..--T........
--------------------------------.ASV.CR.....D-T.TK..A.........
KTVINKDGLTITPAGNGGTTGTNTISBTKDGIK..NKAI..VASGLRAYDDA..DVL...AT...
```

FIG.28G

```
KTVINKDGLTITPAGNGGTTGTNTISBTKDGIK..NKAI..VASGLRAYDDA..DVL...AT...
KTVINKDGLTITPAGNGGTTGTNTISBTKDGIK..NKAI..VASGLRAYDDA..DVL...AT...
------------------------------------------------------------...
------------VNNTIGGSNKQIQVGADGIKFADVNVNVSNAAKFGTTRITEEEIGFAD....
             ** ***   * ** **** *                          * *...
                      ...80        90        100       110       120
                      ...DLNKQNDSVYDGLLNLNEKGTDKSKFLVADETTATVGNLRKL------      33
                      ...----------------------ATDENED..EELEPVQRSV.------      32
                      .....E.HVQDA.K........D.N..S.....N.A.........------      29
                      .....E.HVQDA.K........D.N..S.....N.A.........------      K22
                      .....AR.F.GA..........DAN.N-L..T.DKA.........------      M4071
                      ....AE..VQEA.K........NAS-D.L..E.N.A....D....------      11
                      ..........G.H........N.AN.-.L..D.N.A....D....------      K9
                      ......RHVEDA.K........NAN.QP-..T.S.A....D....------      HSF
                      ......RHVEDA.K........NAN.QP-..TDS.A....D....------      API
                      ......RHVEDA.K........NAN.QP-....S.A....D....------      Rd
                      ...-----KQAP.LDKKQ.KVGSVAITIDNGI.AGNKKIS..A.GSSANDA      4223
                      ....GKVDKK.P.LDKKQ.QVG.VKIT.DSGINAGDQKISNVKDATDDTDA      LES-1
                      ...*         *    **              *       *

       130         140         150         160                    ...
GWVVSTKNSTKEE-SNQVKQADEVLFEG-KDGVTVTSKSENGKHTVT----------------...
R.SFKSAKEGTG.QEGTTEV-------------------------------------------...
...L.S..G.RN.K.Y.........T.-SGAA..S.S.KD....I.----------------...
...L.S..G.RN.K.Y.........T.-SGAA..S.S.KD....I.----------------...
```

FIG.28H

```
...L.S..G.RN.K.Q...H.....-.....----------...
...L.S..G.RN.K.Q...H.....-.G..Q...T.....I.----------...
.....GKEN.K.Q.....K.S.G..Q...T.....AI.----------...
.....G....-.....T.-AGAA.....I.VSVAETKADCGLEKD...
.....G....-.....T.-AGAA.....I.VSVAETKADSGLEKD...
.....G....-.....T.-AGAA.....I.VSVAETKADSGLEKD...
VTIEQL.AAKPTLNAGAGISVTPTEISVDAKSGN..APTY.IGVKT.ELNSDGTSDKFSVKG...
VTYKQL.----------...
                          *  *** * *     *    ** **    * ...

                    ...
                    ...----------                                    33
                    ...----------                                    32
                    ...----------                                    29
                    ...----------                                    K22
                    ...----------                                    M4071
                    ...----------                                    11
                    ...----------                                    K9
                    ...GDTIKLKVDNQNTDNVLTVGNNGTAVTKGGFETVKTGATDADRGKVT   HSF
                    ...GDTIKLKVDNQNTDNVLTVGNNGTAVTKGGFETVKTGATDADRGKVT   API
                    ...GDTIKLKVDNQNTDNVLTVGNNGTAVTKGGFETVKTGATDADRGKVT   Rd
                    ...SGTNNSLVTAEHLASYLNEVNRTADSALQSF-TVKEED-DDDANAIT   4223
                    ...----------QVQQDADGALQSF-SIRDEK-GQEFTISN           LES-1
                    ...* *    *  *    *   *  *    ******   *  *

----------...
```

FIG.28I

```
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
-----------------------------------------------------------...
VKDATANDADKKVATVKDVATAINSAATFVKTENLTTSIDEDNPTDNGKDDALKAGDTLTFK...
VKDATANDADKKVATVKDVATAINSAATFVKTENLTTSIDEDNPTDNGKDDALKAGDTLTFK...
VKDATANDADKKVATVKDVATAINSAATFVKTENLTTALDEADAKDQG-DDALKAGDTLTFK...
VAKDTTKNAGAVSILKLKGKNGLTVATKKD-GTVTFGLSQDSGLTIGKSTLNNDGLTVKDTN...
LYSNGNTPNTFETITFA-GENGISISNDIAKGKVKVGIDPINGLTTPKLTVGSDKDGKTQLV...
*   *                **  *    **    *                          ...

...----------------------------------------------   33
...----------------------------------------------   32
...----------------------------------------------   29
...----------------------------------------------   K22
...----------------------------------------------   M4071
...----------------------------------------------   11
...----------------------------------------------   K9
...AGKNLKVKRDGKNITFDLAKNLEVKTAKVSDTLTIGGNTPTGGTTAT--   HSF
...AGKNLKVKRDGKNITFDLAKNLEVKTAKVSDTLTIGGNTPTGGTTAT--   API
...AGKNLKVKRDGKNITFDLAKNLEVKTATFSDRLTIG-------------   Rd
...-EQIQVGANGI.FTNVNGSNPGTGIANTARITRDKIGFAGSDGAVDTNK   4223
...IEQVASGN-.T...IR---------------------------------   LES-1
           *    * *      *         * * * ** *
...
```

FIG.28J

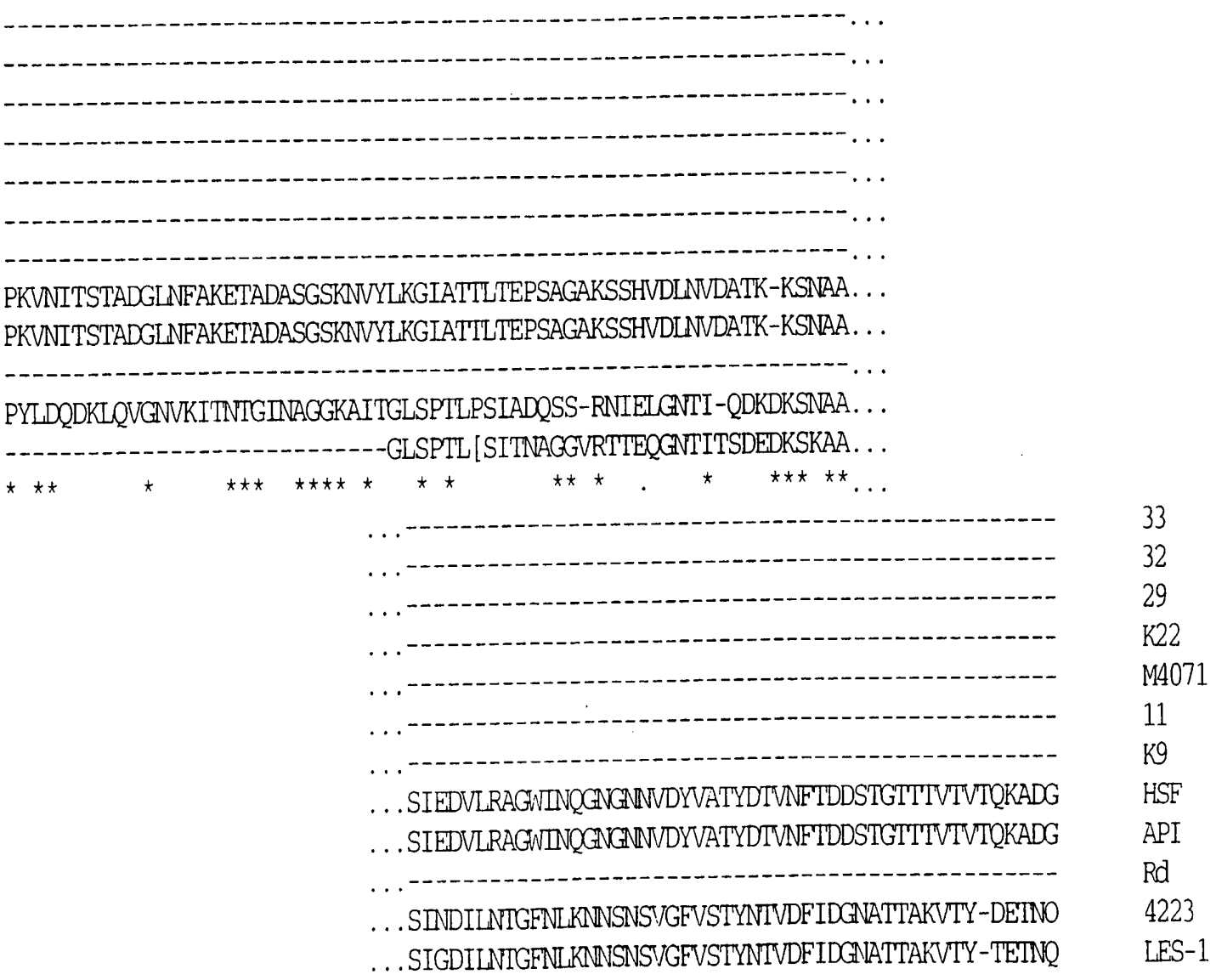


FIG.28K

```
                                          ...** *** *****  * * * ****** *** *  **** ***  * **

--------------------------------------------------------------...
--------------------------------------------------------------...
--------------------------------------------------------------...
--------------------------------------------------------------...
--------------------------------------------------------------...
--------------------------------------------------------------...
--------------------------------------------------------------...
KGADVKIGAKTSVIKDHNGKLFTGKDLKDANNGATVSEDDGKDTGTGLVTAKTVIDAVNKSG...
KGADVKIGAKTSVIKDHNGKLFTGKDLKDANNGATVSEDDGKDTGTGLVTAKTVIDAVNKSG...
--------------------------------------------------------------...
TSKVVYDVNVDDTTIHLTGTDDNK-KLGVKTTKLNKTDANGNTATNFNVNSSDEDALVNAKD...
TSKVTYDVNVDEKTIELTGDNGKTNKIGVKTTTLTTINANGK-ATNF-STTDNDALVNAKDI...
 *                *                 * **  **  * *     **      ...
                                          ...---------------------------------------------------   33
                                          ...---------------------------------------------------   32
                                          ...---------------------------------------------------   29
                                          ...---------------------------------------------------   K22
                                          ...---------------------------------------------------   M4071
                                          ...---------------------------------------------------   11
                                          ...---------------------------------------------------   K9
                                          ...WRVTGEGATAETGATAVNAGNAETVTSGTSVNFKNGNATTATVSKDNGNIN   HSF
                                          ...WRVTGEGATAETGATAVNAGNAETVTSGTSVNFKNGNATTATVSKDNGNIN   API
                                          ...---------------------------------------------------   Rd
```

FIG.28L

```
...IAENLNTLAKEIHTTKGTADTALQTFTVKKVDENNNADDANAIT-------  4223
...AENLNTLAKEIHTTKGTADTALQTFKVKK-----DGATDDETIT-------  LES-1
       ** * *  **   *  *             *      ***
...

-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
VKYDVNVGDGLKIGDDKKIVADTTTLTVTGGKVSVPAGANSVNNNKKLVNAEGLATALNNLS...
VKYDVNVGDGLKIGDDKKIVADTTTLTVTGGKVSVPAGANSVNNNKKLVNAEGLATALNNLS...
-------------------------------------------------------...
VGQKNANNQ--VNTLTLKGENGLNIKTDKNGTVTFGIN-----------------...
VGKDGTQNGKTVNTLKLKGENGLTVATNKDGTVTFGIN-----------------...
*           *   *       *   * **                       ...
...----------------------------------------------------  33
...----------------------------------------------------  32
...----------------------------------------------------  29
...----------------------------------------------------  K22
...----------------------------------------------------  M4071
...----------------------------------------------------  11
...----------------------------------------------------  K9
...WTAKADKYADGESEGETDQEVKAGDKVTF-KAGKNLKVKQSEKDFTYSLQD  HSF
```

FIG.28M

```
...WTAKADKYADGESEGETDQEVKAGDKVTF-KAGKNLKVKQSEKDFTYSLQD   API
...-------------------------------------------------   Rd
...---------------TTSGLKAGKST-LNDGGLSIKNPTGSEQIQVGADG   4223
...---------------TQSGLKAGDSTTLNKDGKSIKNPASNEQIQVGADG   LES-1
...                  *   ****       *  **   *  *   * *
```

```
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
-------------------------------------------------------...
TLTGLTSITLGGTANGRNDTGTVINKDGLTITLANGAAAGTDASNGNT----ISVTKDGISA...
TLTGLTSITLGGTANGRNDTGTVINKDGLTITLANGAAAGTDASNGNT----ISVTKDGISA...
-------------------------------------------------------...
VKFAKVNNNGVVGAGIDGTTRITRDEIGFTGTNGSLDKSKPHL-----------SLDGINA...
VKFAKVDK-GNSSTGIDGTSRITKDQIGFTGANGSLDTTKPHLTKDKLKVGEVEITNTGINA...
   *        **     *    * * * * *    *    *        * **  ** *...
```

```
...------------------------------   33
...------------------------------   32
...------------------------------   29
...------------------------------   K22
...------------------------------   M4071
...------------------------------   11
```

FIG.28N

```
...------------------------------                    K9
...GNKEITNVKSA---------LKTYKDTQNTA                   HSF
...GNKEITNVKSA---------LKTYKDTQNTA                   API
...------------------------------                    Rd
...GGKKITNIQSGEIAQNSHDAVTGGKIYDLKT                   4223
...GGKKITNIQSGDITQNSNDAVTGGRVYDLKT                   LES-1
...* * **** ******** *** *    *  *

------------------------------------------------------...
------------------------------------------------------...
------------------------------------------------------...
------------------------------------------------------...
------------------------------------------------------...
------------------------------------------------------...
------------------------------------------------------...
------------------------------------------------------DE...
GATQPAANTAEVAKQDLVDLTKPATGAAGNGADAKAPDTTAATVGDLRGLGWVLSAKKTADE...
------------------------------------------------------...
------------------------------------------------------EL...
------------------------------------------------------EL...
                                                      * ...

...------------------------------                     33
...------------------------------                     32
...------------------------------                     29
...------------------------------                     K22
```

FIG.28O

```
...------------------------------                                M4071
...------------------------------                                11
...------------------------------                                K9
...TQDKEFHAAVKNANEVEFVGKNGATVSAKT                                HSF
...TQDKEFHAAVKNANEVEFVGKNGATVSAKT                                SPI
...------------------------------                                Rd
...ENKISSTAKTAQNSLHEFSVADEQGNNFTV                                4223
...ESKINSAAKTAQNSLHEFSVADEQGNNFTV                                LES-1
...          *    *    **   *  *

--------------------------------------------------------------...
--------------------------------------------------------------...
-------------------------------------------------ISVTKGSFAEVKT...
-------------------------------------------------ISVTKGSFAEVKT...
--------------------------------------------------------------...
--------------------------------------------------------------...
--------------------------------------------------------------...
DNNGKHTVTIDVAEAKVGDGLEKDTDGKIKLIVDNTDGNNLLTVDATKGASVAKGEFNAVTT...
DNNGKHTVTIDVAEAKVGDGLEKDTDGKIKLIVDNTDGNNLLTVDATKGASVAKGEFNAVTT...
SNPYSSYDTSKTSDVITFAGENGITTKVNKGVVRVGIDQTKGLTTPKLTVGNNNGKGIVIDS...
SNPYSSYDTSKTSDVITFAGENGITTKVNKGVVRVGIDQTKGLTTPKLTVGNNNGKGIVIDS...
*      *   **     * * *   * * * *         **      * * *...

...-----------------------------------------                     33
...-----------------------------------------                     32
...DATTGGQVNAD-RGKVK----AEDENGADVDKKV-------                     29
```

FIG.28P

```
...DATTGGQVNAD-RGKVK----AEDENGADVDKKV-------          K22
...-----------------------------------------          M4071
...-----------------------------------------          11
...-----------------------------------------          K9
...DATTAQGTNANERGKVVVKGSNGATATETDKKKV-------          HSF
...DATTAQGTNANERGKVVVKGSNGATATETDKKKV-------          API
...QNGQNTITGLSNTLANVTNDKGSVRTTEQGNIIKDEDKTRA          Rd
...KDGQNTITGLSNTLANVTNDGAGHSLS-QGLAN-DTDKTRA          LES-1
                                  *
...

                                                             ...
-------------------------------------------------------------...
                         INLNTDSSGNAVGSSTITFKAGDNLKIKQSGN...
ATVKDVAKAINDAATFVKVESTDDDIENGAAGKNETTDQALKAGDTLTLKAGKNLKAKLDQN...
ATVKDVAKAINDAATFVKVESTDDDIENGAAGKNETTDQALKAGDTLTLKAGKNLKAKLDQN...
-------------------------------------------------------------...
-------------------------------------------------------------...
-------------------------------------------------------------...
ATVGDVAKAINDAATFVKVEN-DDSATIDDSPTDDGANDALKAGDTLTLKAGKNLKVKRDG-...
ATVGDVAKAINDAATFVKVEN-DDSATIDDSPTDDGANDALKAGDTLTLKAGKNLKVKRDG-...
-------------------------------------------------------------...
ASIVDVLSAGFNLQGNGEAVDFVSTYDTVNFADGNATTAKVTYDDTSKTSKVVYDVNVDDTT...
ASIGDVLNAGFNLQGNGEAVDFVSTYDTVDFIDGNATTAKVTYDDTSKTSKVVYDVNVDNKT...
*** *      *    *                   *           *
                    ...        170        180        190        200
```

FIG.28Q

```
...-----FALANDLNVKNATVSDKLSLGANGKKVDITSDANG-------     33
...D--FTYS.KKE.KNLTSVETE...F....N.........-------     32
...GKSVT....K..D.TS.K......I.KDTN..........-------     29
...GKSVT....K..D.TS.K......I.KDTN..........-------     K22
...-----.T.EK..................N.......T..-------     M4071
...-----....K..G..T.....T.TI.GGAAAGAT.TPKVNVTSTTDG     11
...-----....K..SMRT.....T.TI.GSTTTGSA.TPKVNVTSTASG     K9
...-KNIT.......S..S..........T..N..N....TK.-------     HSF
...-KNIT.......S..S..........T..N..N....TK.-------     API
...-------------------------...N.......T..-------     Rd
...IEVK-DKKLGVKTTTLTSTGTGANKFALSNQATGDALVKASDIVA--     4223
...IEVTSDKKLGVKTTTLTKTSANGNATKFSA-ADGDALVKASDIAT--     LES-1
...                 *  *        *      *

       210        220       230       240        250        ...
LKFAKQGT-NGQNGN--VHLNGIASTLDDPRVGGKTAHLTKEISDTERN--RAASVGDVLNA...
..L..T.NG....S.--..........T.TLA.T.G.VDTN.DAVNYH--......Q....S...
..L..T.NG......--..........T.TIT.MT.QASNGVAVQ-NH--......A........
..L..T.NG......--..........T.TIT.MT.QASNGVAVQ-NH--......A........
.....PS.-......--..........T.TIT.TTKSATNGVDVQNH-.--.....A........
.....DAA--.A..DTT......G...T.TK..SPAT.IDGGDQS.HYT--....IK........
.V...GA.GANGDTT--...TN.....Q.TLLNTGVVSKLDGNGITADEKK.....Q....S...
.....DSKT-.DDA.--I.........T.TLLNSGATTNLGGNGITDNEKK.....K........
..x..DSKT-.DDA.--I.........T.TLLNSGATTNLGGNGITDNEKK.....K........
.....P..-......--..........................--..............
```

FIG.28R

```
------------------...TLSGDIQTAKGASQANNSAGYVDADGNKVIYDSTDNKYYQA...
------------------...TLSGDIQTAKGASQASSSASYVDADGNKVIYDSTDNKYYQV...
*  ***      **   ***  *** *                          **     * ...
                          ...260         270        280        290        300
                          ...GMNIRGAK--TIGG-TVDNVDFVSTYDTVEFASGANANVSVTTDDN--      33
                          .......Q.NGNNVDFVR.Y.T....N---------.........A.TAH-      32
                          .......Q.NGASVDFVNAY.T....N---------.T.T..N....TAH-      29
                          .......Q.NGASVDFVNAY.T....N---------.T.T..N....TAH-      K22
                          .......Q.NGAS---------....N.....D.VN.L.T..N....TAHN      M4071
                          .......K.V.AGSTT-GQSE.....H.......L.-.DTETTTV.V.S--      11
                          .......K.V.TGAT---.S......R.......L..SEETTL..V.S---      K9
                          ......V..V.PASANNQ-.E.I...A.....D.V..DKDTT...VES---      HSF
                          ......V..V.PASANNQ-.E.I...A.....D.V..DKDTT...VES---      API
                          ..........--....-.............................--        Rd
                          ...KNDGTVD.TKEVAKDKLVAQAQTPDGTLAQMNVKSVI.KEQVN.A.--      4223
                          ...NDKGQVD.NKEVAKDKLVAQAQTPDGTLAQMNVKSVI.KEQVN.A.--      LES-1
                          ...                                    *

      310        320        330        340        350        360 ...
KKTTVRVDVTGLPVQYVTEDSKTVVKVGNEYYEAKQDGSADMDKKV-ENGKLAKTKVKLVSA...
....................G..........K..D......NQ..-...E.............
....................G......D.K.............-...E.............
....................G......D.K.............-...E.............
....................GE.......................-................
            ..F......G.......................-K..E.V............
```

FIG.28S

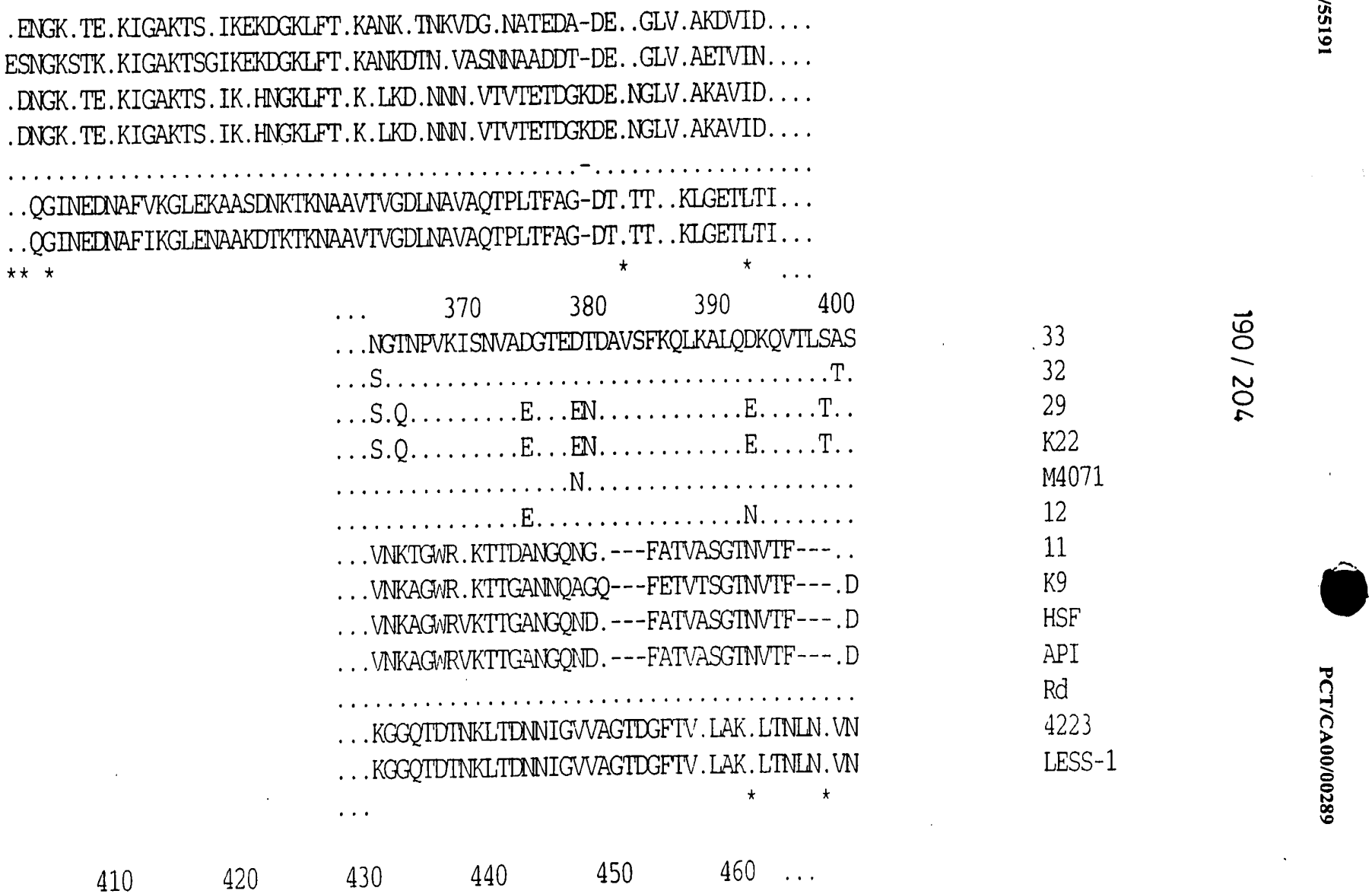


FIG.28T

```
NAYANGGSDADGGKATQTLGNDLNFKFKSTDSELLNIKAAGDTVTFTPKKGSVQVGDDGKAT...
.......T.N.........S.G.......S.G...K.S.T.....................S...
.......N...........N.G.........G......VEN..............E........
.......N...........N.G.........G......VEN..............E........
..............GI...S.G.........G.......EN.......................
...........V..V....S.G.........G.......DK....I.---------------...
GNGTTATVTNG-TDGITVKYDAKVGDGLKLDGD-KIAADTTALTVNDGKNANNPKGKVADVA...
GNGTTAVVTGDATNGITVKYEAKVGDGLKIGNDQKITADTTALTVTGGK--------VTAPD...
GNGTTAEVTKANDGSITVKYNVKVADGLKLDGD-KIVADTTVLTVADGK--------VTAPN...
GNGTTAEVTKANDGSITVKYNVKVADGLKLDGD-KIVADTTVLTVADGK--------VTAPN...
..............G....S.G.........G.......EN.......................
AGGTKIDDKGVSF-----------------------------------------------...
AGGTRIDEKGISFVDANGQAKANTPVLSANGLDLGGKRISNIGAAVDDNDAVNFKQFNEVAK...
   *                              *                *          ...
```

```
...      470        480        490          500
...IQDGAKTTTGLVEASELVDSLNKLGWKVGVGKDGTG---AT      33
....SK..N..E.........E...........E.V.S.---EL      32
.....N.T...D.........E...........D...S.---EL      29
.....N.T...D.........E...........D...S.---E.      K22
.................................T.T....V.---      M4071
...-----------------------------------------      12
...STDEKK----..T.KG..TA..S.S.TTTAAEADG.---TL      11
...ATNGKK----..N..G.A.A....S.TAK-AEADTANGGEL      K9
...NGDGKK----F.D..G.A.A....S.TATA..E...---EV      HSF
...NGDGKK----F.D..G.A.A....S.TATA..E...---EV      API
```

FIG.28U

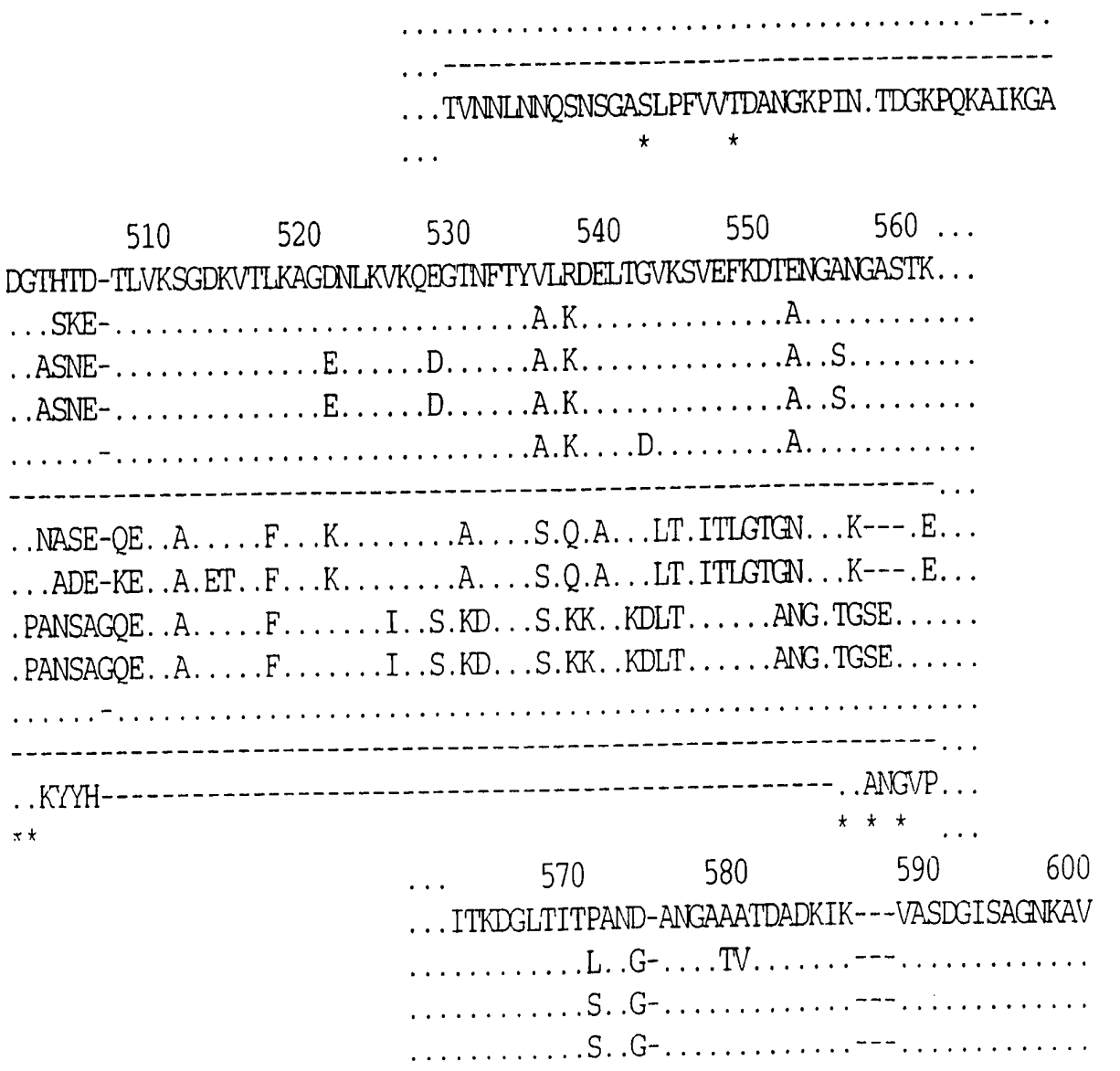


FIG.28V

```
...............G-.GA.G.NT.NT.S---.TK..........     M4071
...---------R.SG-.............---.............     12
....N..........G-.---G.NN.NT.S---.TK......DQS.     11
....N..........G-----G.NN.NT.S---.TK......DQS.     K9
...............G-.GA.G.NT.NT,S---.TK..........     HSF
...............G-.GA.G.NT.NT,S---.TK..........     API
..........F....G-.............---......R......     Rd
...-------------------------------------------     4223
...VD...KP..D.DKL..L..HGKPLDAGHQV...L.-GNSD-.I     LES-1
...* ***  ** *   *  **          **  * * *  **

     610       620       630       640       650       660 ...
KNVVSGLKKFGDANFNPLTSSADNLTKQYDNAYKGLTNLDEKSKGKQTPTVADNTAATVGDL...
--------------------------------------------------------------...
..............................D..........GAD...L.................
..............................D..........GAD...L.................
...............D..................................................
.-------------------------------------------------------------...
...............D...........N.D..........GTD....V.................
..............................D..........GAD...L.................
T..........GHTLANGTV..FE-.H.......D.....GADNN-...................
T..........GHTLANGTV..FE-.H......XD.....GADNN-...................
--------------------------------------------------------------...
--------------------------------------------------------------...
-----------------TLTNIKSTLP.I.TPNT.NA.AGQAQSLPSLSAAQQSN..S.K.V...
```

FIG.28W

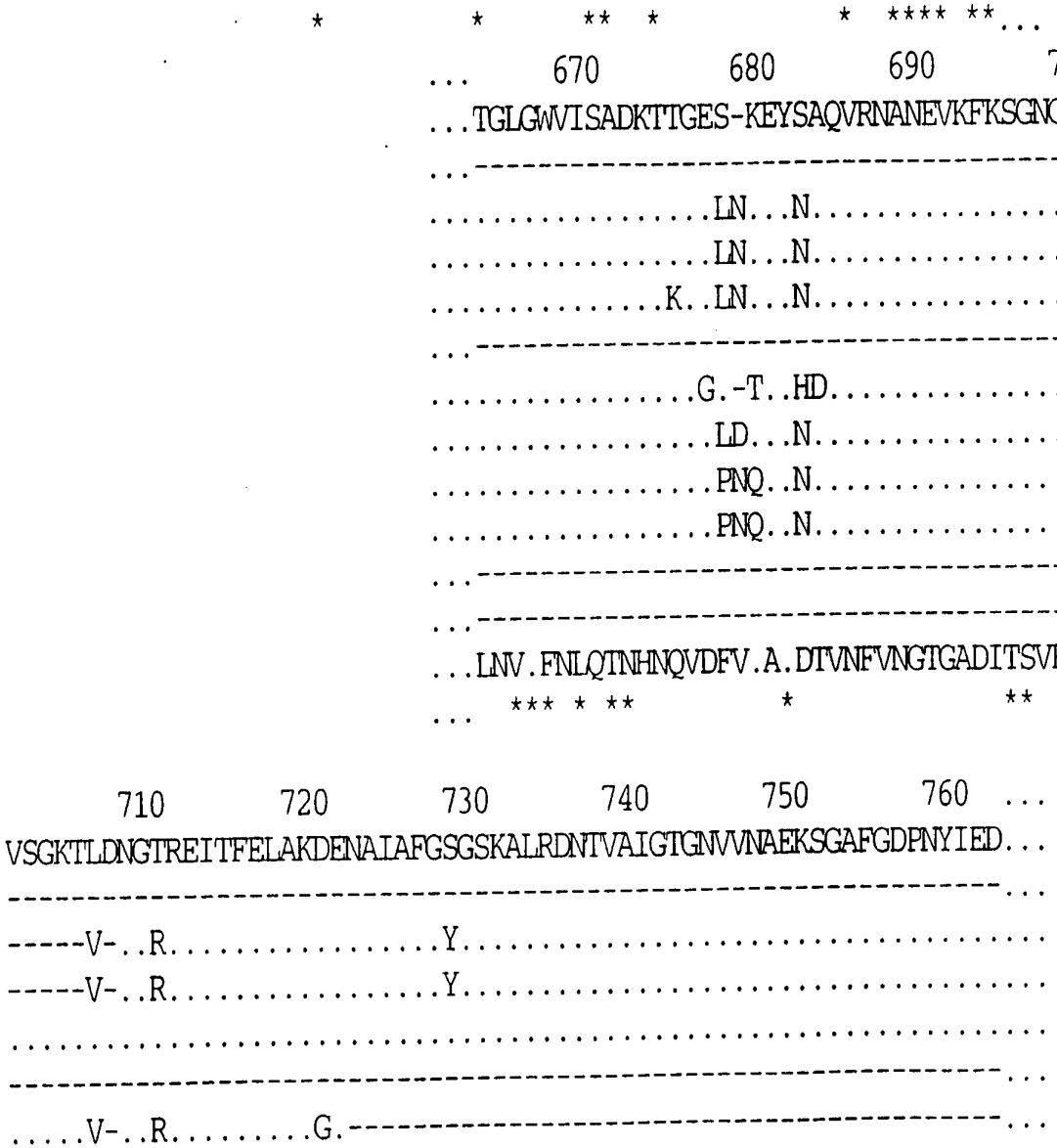


FIG.28X

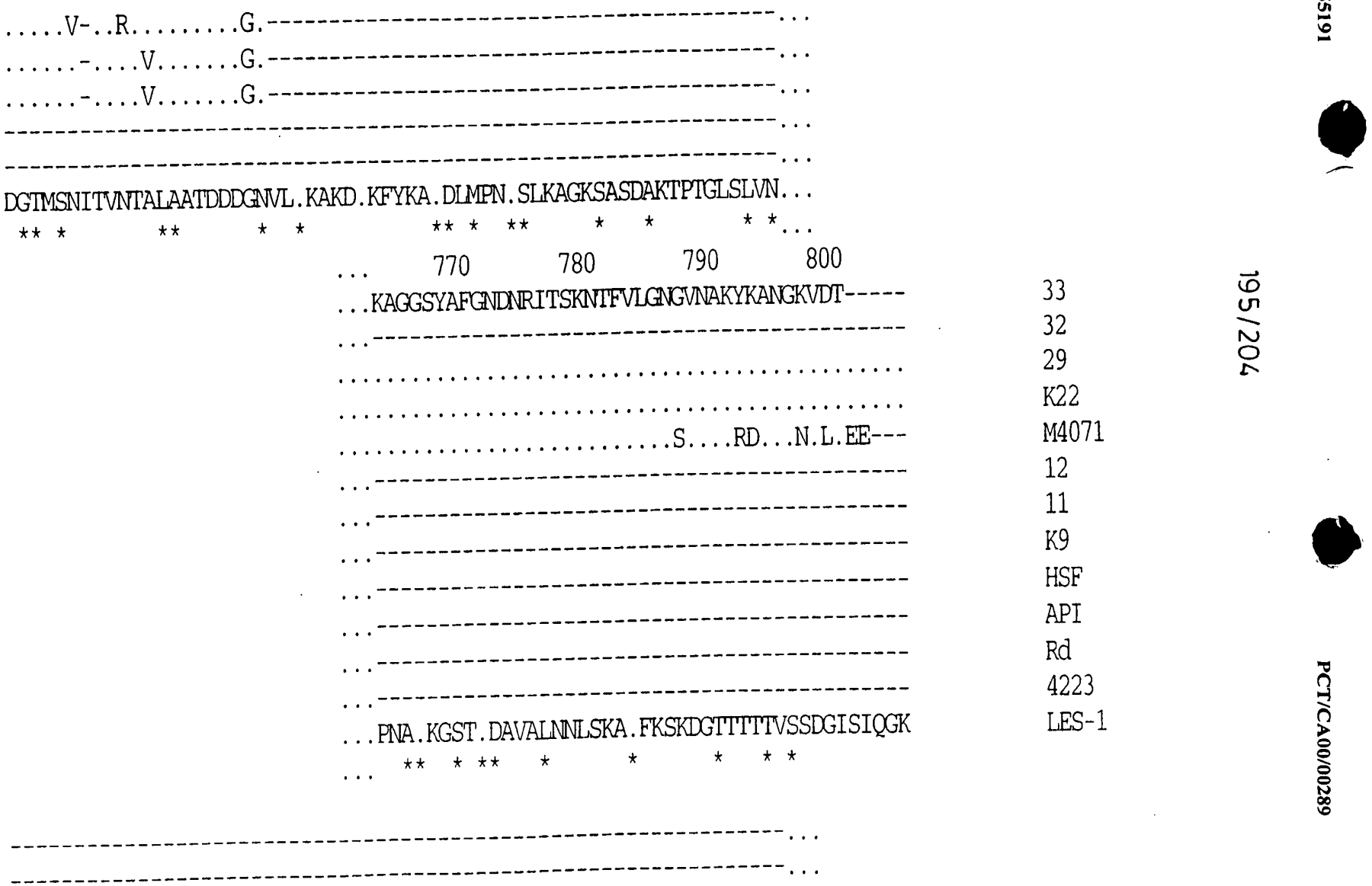


FIG.28Y

```
-----------------------------------------------------------------...
-----------------------------------------------------------------...
-----------------------------------------------------------------...
-----------------------------------------------------------------...
VVKSNEFTVKETNGKETSLVKVGDLYYSKEDIDLTTGQPKLKDGNIVAAKYQDKGGKVVS-V...
VVKSNEFTVKETNGKETSLVKVGDLYYSKEDIDPATGKPKVTNGNAVAAKYQDKDGKVVSAD...
VVKSNEFTVKNADGSETNLVKVGDLYYSKEDIDPATSKPMTGKTE----KYKVENGKVVSAN...
VVKSNEFTVKNADGSETNLVKVGDLYYSKEDIDPATSKPMTGKTE----KYKVENGKVVSAN...
-----------------------------------------------------------------...
VDSSGQAKANTPVLSANGLDLGGKVISNVGKGTKDTDAANVQQLNEVRNLLGLGNAGNDNAD...
DNSSI-------TLSKDGLNVGGKVISNVGKGTKDTDAANVQQLNEVRNLLGLGNAGNDNAD...
 * *            *   *           *      * *       *    **...

...---------------------------------------  33
...---------------------------------------  32
...---------------------------------------  29
...---------------------------------------  K22
...---------------------------------------  M4071
...---------------------------------------  12
...TDNTEATITNKGSGYVTGNQ-------------------  11
...GSSNTAVTLTNKGYGYVTGNQ------------------  K9
...GSK-TEVTLTMKGSGYVTGNQ------------------  HSF
...GSK-TEVTLTMKGSGYVTGNQ------------------  API
...---------------------------------------  Rd
...GNQVNIADIKKDPNSGSSSNRTVIKAGTVLGGKGNNDT  4223
...GNQVNIADIKKDPNSGSSSNRTVIKAGTVLGGKGNNDT  LES-1
```

FIG.28Z

```
                              ...        *      ***

----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
VADAIAKSGFEKGKADEADAKRAFDD--KTKALSAGTTE-IVNAHDKVRFANGLNTKVSAAT...
VADAIAKSGFEKGKADAEKAKAAFGD--ETKALSSDKLE-TVNANDKVRFANGLNTKVSAAT...
VADAIAKSGFEKGKADAAEAEKAFAESAKDKQLSKDKAE-TVNAHDKVRFANGLNTKVSAAT...
VADAIAKSGFEKGKADAAEAEKAFAESAKDKQLSKDKAE-TVNAHDKVRFANGLNTKVSAAT...
----------------------------------------------------------...
EKLATGGIQVGVDKDGNANGDLSNVWVKTQKDGSKKALLATYNAAGQTNYLTNNPAEAIDRI...
EKLATGGVQVGVDKDGNANGDLSNVWVKTQKDGSKKALLATYNAAGQTNYLTNNPAEAIDRI...
  * *    *     * *    * *    * **    *     *    ...
                   ...-------------------------------------   33
                   ...-------------------------------------   32
                   ...-------------------------------------   29
                   ...-------------------------------------   K22
                   ...-------------------------------------   M4071
                   ...-------------------------------------   12
                   ...VESTDANGDKVTTTFVKTDVELPLTQIYNTDANGKKITKVV   11
                   ...VESTDANGDKVTTTFVKTDVELPLTQIYNTDANGKKI---V   K9
                   ...VESTDANGDKVTTTFVKTDVELPLTQIYNTDANGNKI---V   HSF
```

FIG.28A'

```
...VESTDANGDKVTTTFVKTDVELPLTQIYNTDANGNKI---V   API
...-----------------------------------------   Rd
...NEQGIRFFHVNDGNQEPVVQGRNGIDSSASGKHSVAIGFQ-   4223
...NEQGIRFFHVNDGNQEPVVQGRNGIDSSASGKHSVAIGFQ-   LES-1
... * *        *            *   *   *  **

----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
----------------------------------------------------------...
KDGQTKWYELNADGTADMTKEVTLGNVDSDGKKVVKDNDG---KWYHAKADGTADKTKGEVD...
KNGD-KWYYTKDDGSTDMTKEVTLGNVDSDGKKVVKEDN----KWYGVKSDGSTDKTQVVEE...
KKADGKWYELNADGTASN-KEVTLGNVDANGKKVVKVTENGADKWYYTNADGAADKTKGEVS...
KKADGKWYELNADGTASN-KEVTLGNVDANGKKVVKVTENGADKWYYTNADGAADKTKGEVS...
----------------------------------------------------------...
AKADGEAAVAIGRQTQAGNQSIAIGDNAQATGDQSIAIGTGNVVAGKHSGAIGDPSTVKADN...
AKADGEAAVAIGRQTQAGNQSIAIGDNAQATGDQSIAIGTGNVVTGKHSGAIGDPSTVKADN...
  **        *     *****    *         *        *  *   *     ...
```

```
...-------------------------   33
...-------------------------   32
...-------------------------   29
...-------------------------   K22
...-------------------------   M4071
```

FIG.28B'

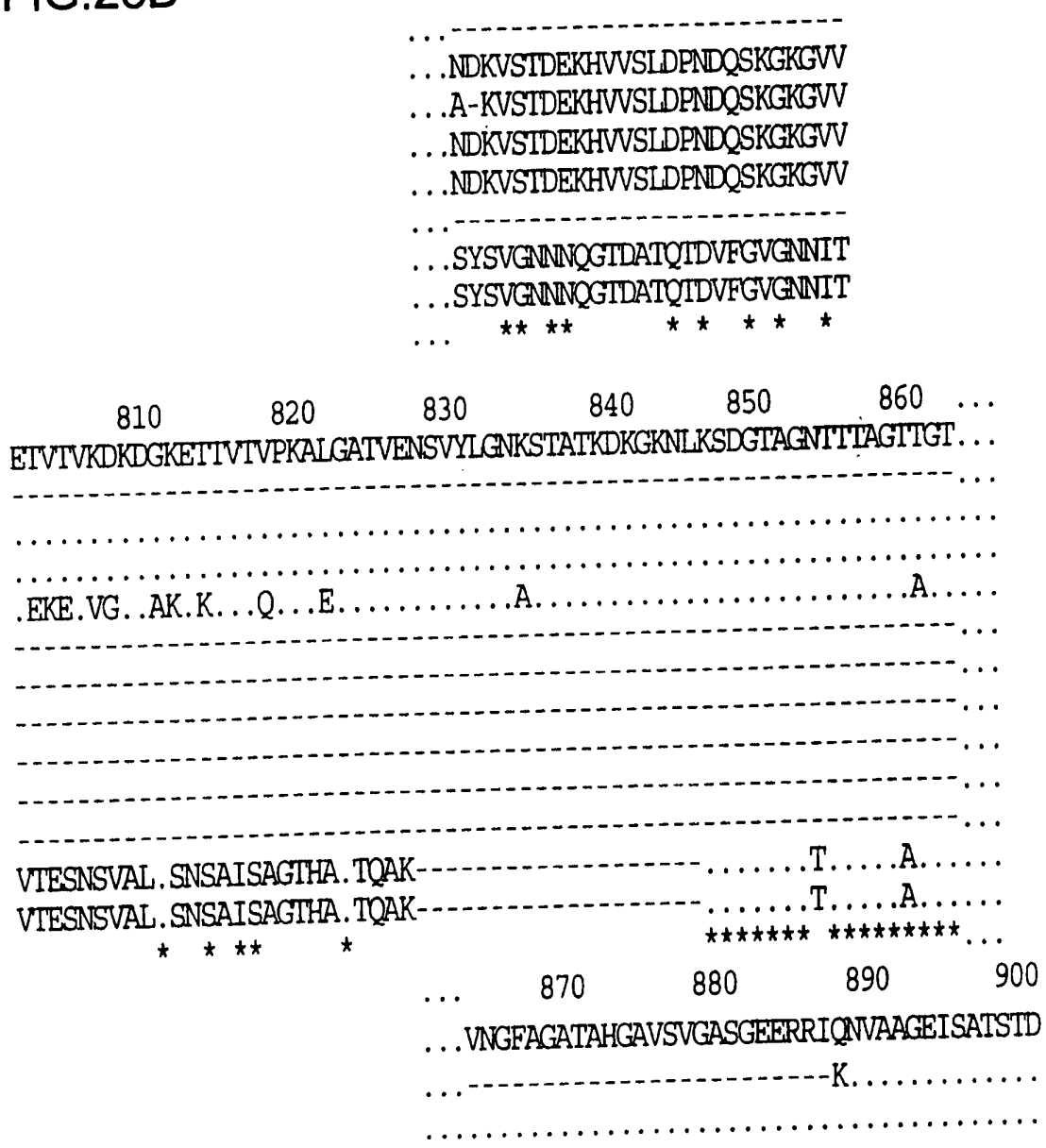


FIG.28C'

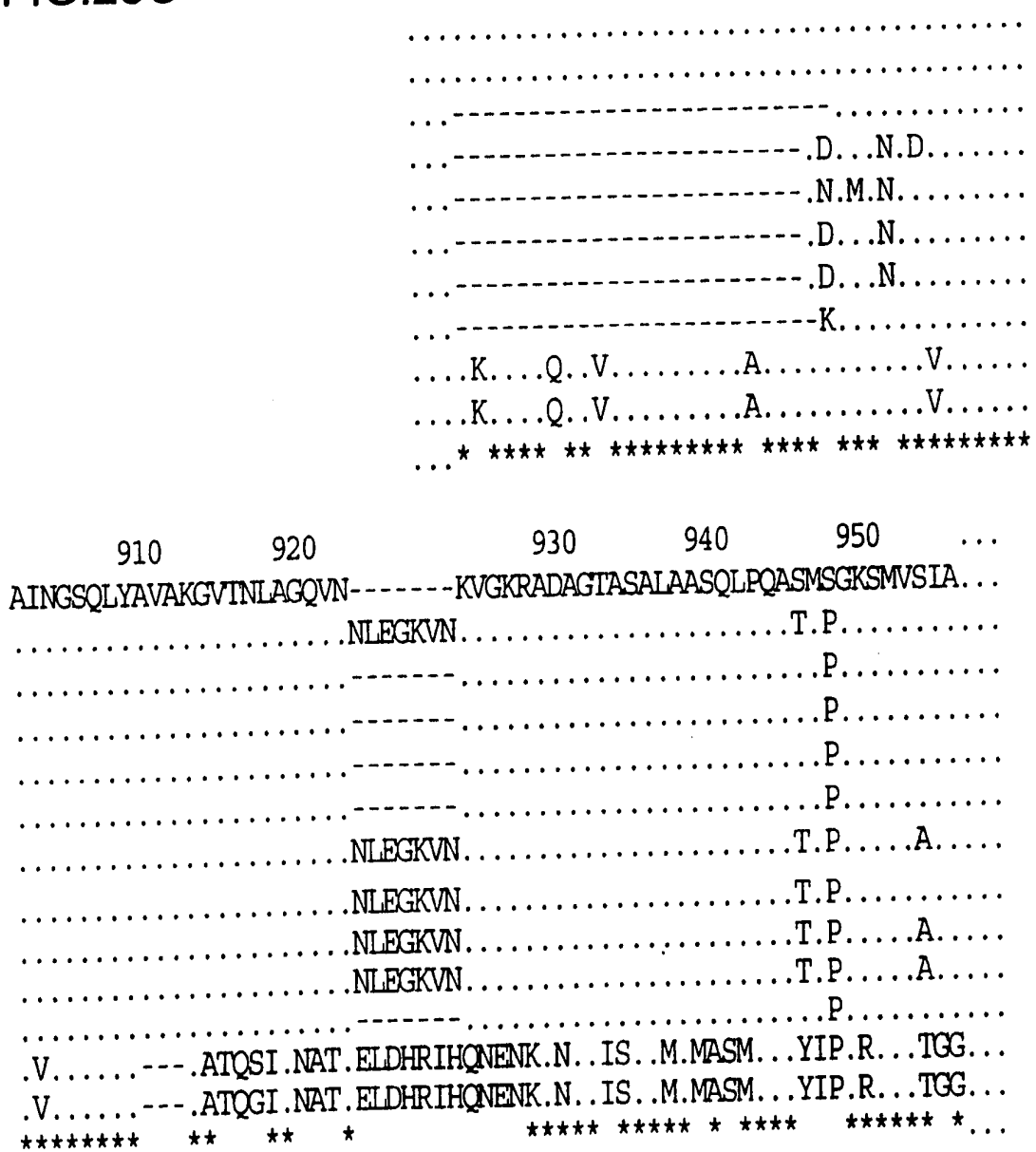


FIG.28D'

```
...  960       970       980       990       1000
...GSSYQGQSGLAIGVSRISDNGKVIIRLSGTTNSQGKTGVAAGVGYQW*      33
..........N.....................................*      32
..........N.....................................*      29
..........N.....................................*      K22
................................................*      M4071
.......................L........................*      12
..........N.....................................*      11
..........N.....................................*      K9
..........N.....................................*      HSF
..........N.....................................*      API
................................................*      Rd
...IATHN..GAV.V.L.KL....QWVFKIN.SADT..HV.A.V.A.FHF*    4223
...IATHN..GAV.V.L.KL....QWVFKIN.SADT..HV.A.V.A.FHF*    LES-1
...  ****** ************* * ** ******* * * * ** *
```

FIG.29

Oligonucleotides primers to PCR amplify truncated strain 11 S44 hia gene.

```
         Nde I
           M  S44A  T  V  E  A  N  N  N  T                          SEQ ID NO:56
5'   GGGAATTCATATGTCCGCAACGGTTGAGGCGAACAACAATACT   3'    6817.SL    SEQ ID NO:55

                         Sty I
     H  T  I  T  F  A  L  A  K  D  L  G                             SEQ ID NO:59
     CACACCATTACCTTTGCTTTAGCGAAAGACCTTGGT                           SEQ ID NO:58
3'   GTGTGGTAATGGAAACGAAATCGCTTTCTGGAACCACCCTAGGGC   5'  6818.SL    SEQ ID NO:57
```

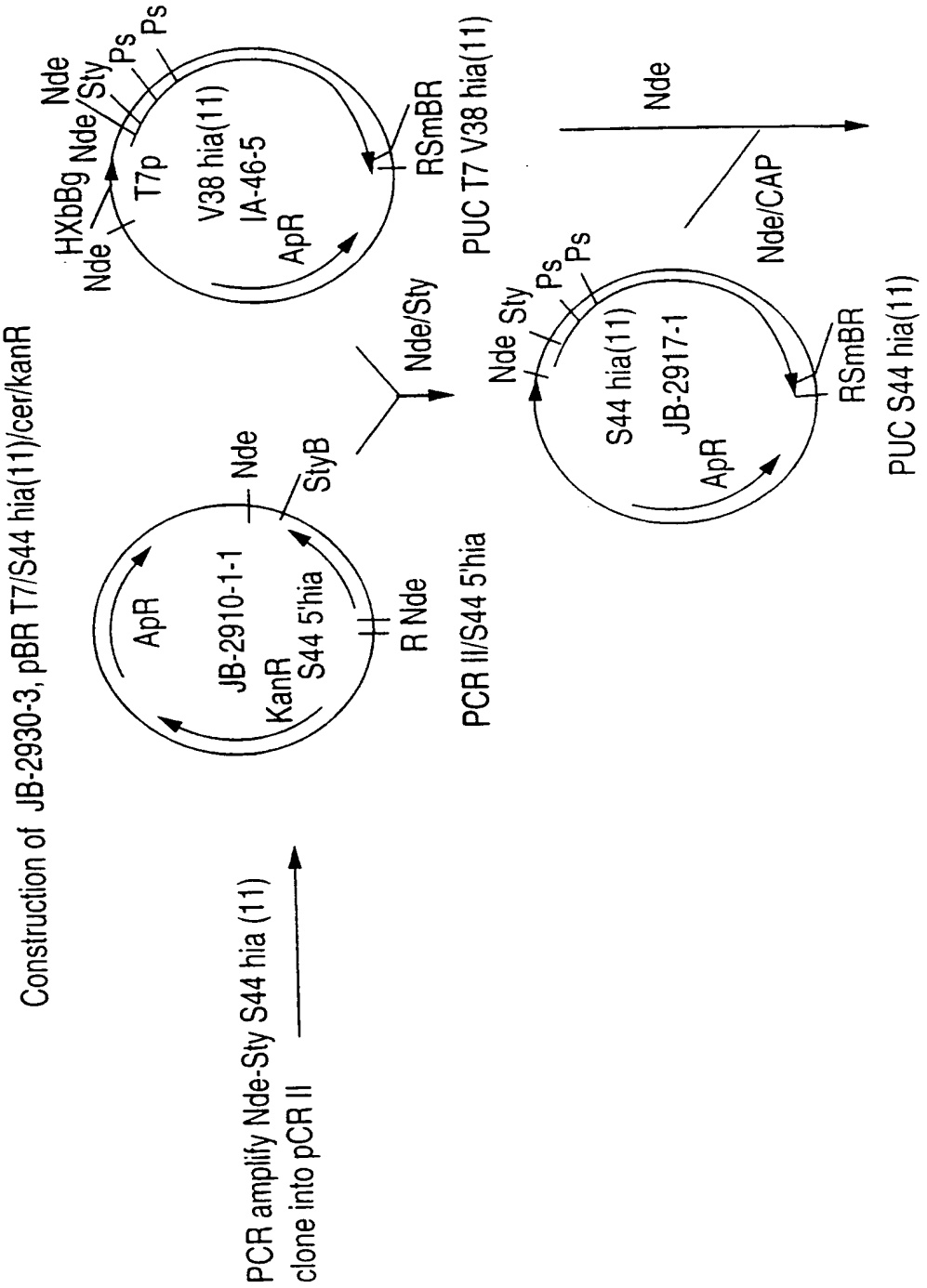

Construction of JB-2930-3, pBR T7/S44 hia(11)/cer/kanR
PCR amplify Nde-Sty S44 hia (11)
clone into pCR II
JB-2910-1-1
ApR
KanR
S44 5'hia
Nde
StyB
R Nde
PCR II/S44 5'hia
Nde/Sty
HXbBg
Nde
Nde
Sty
Ps
Ps
T7p
V38 hia(11)
IA-46-5
ApR
RSmBR
PUC T7 V38 hia(11)
Nde
Nde/CAP
Nde Sty
Ps
Ps
S44 hia(11)
JB-2917-1
ApR
RSmBR
PUC S44 hia(11)

FIG.30A

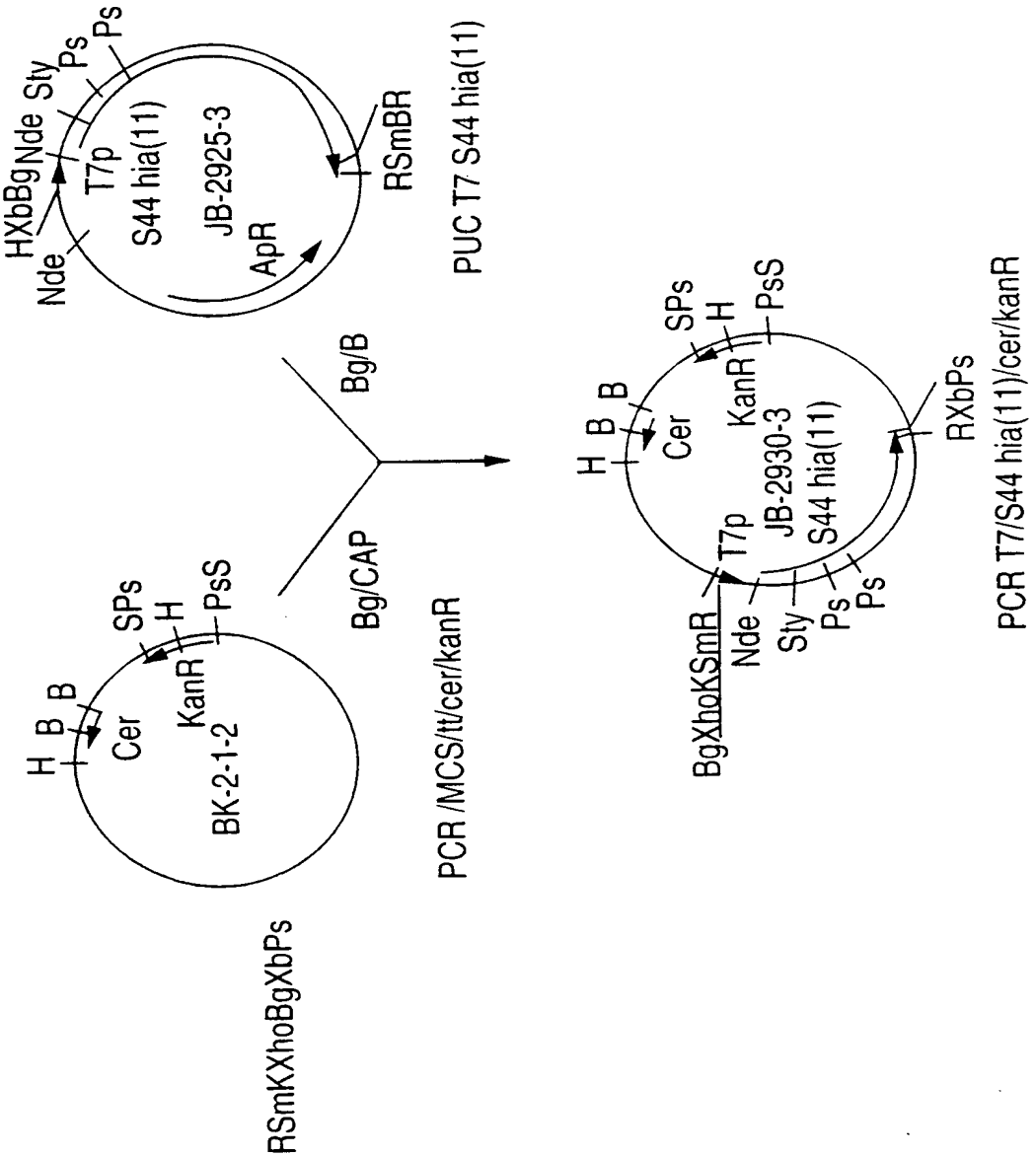
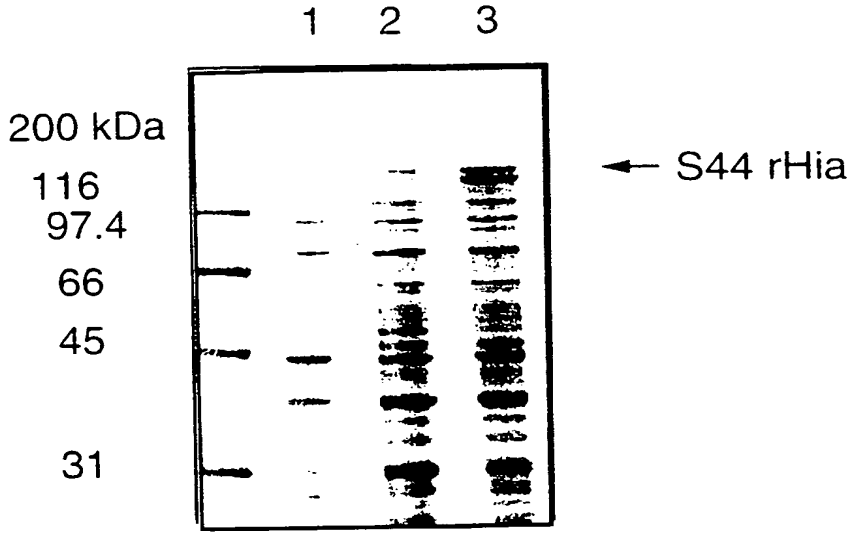

HXbBgNde Sty Ps Ps
Nde
T7p
S44 hia(11)
JB-2925-3
ApR
RSmBR
PUC T7 S44 hia(11)
Bg/B
Bg/CAP
PCR /MCS/tt/cer/kanR
H B B
Cer
SPs
H
KanR
PsS
BK-2-1-2
RSmKXhoBgXbPs
BgXhoKSmR
Nde
T7p
JB-2930-3
S44 hia(11)
Sty
Ps
Ps
RXbPs
PCR T7/S44 hia(11)/cer/kanR

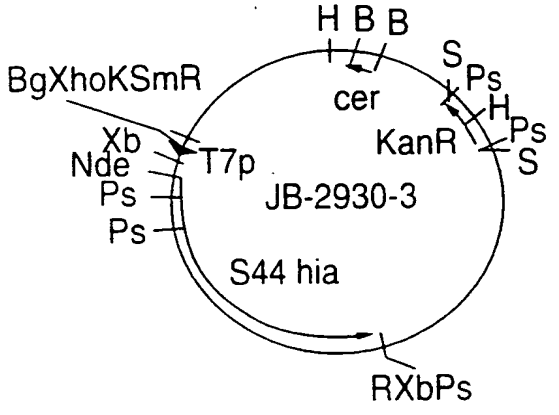
FIG.30B

Production of S44 rHia from different vectors

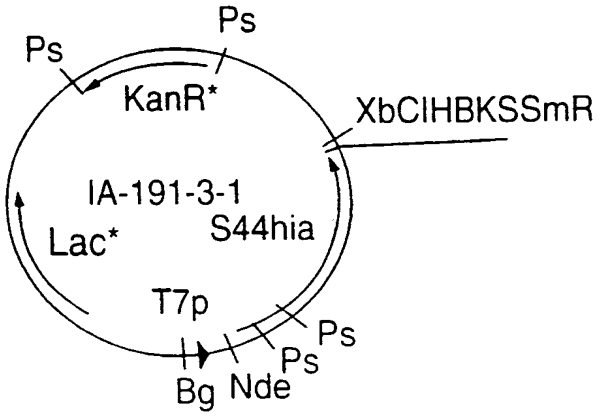


1. pET S44 hia t_0
2. pET S44 hia t_4
3. pBR T7 S44 hia/cer/kanR t_4

FIG.31



pBR T7 S44 hia/cer/KanR



pET S44 hia

FIG.32

INTERNATIONAL SEARCH REPORT

Int[illegible]al Application No
CA 00/00870

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C07K14/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SASAKI K ET AL: "Control mechanism of the 200 kD protein gene expression in Moraxella (Branhamella) catarrhalis." ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR, vol. 99, 1999, page 89 XP000971104 99th General Meeting of the American Society for Microbiology;Chicago, Illinois, USA; May 30-June 3, 1999, 1999 ISSN: 1060-2011 abstract B/D-306 ---	1,2
X	WO 96 34960 A (LOOSMORE SHEENA M ;CONNAUGHT LAB (CA); CHONG PELE (CA); KLEIN MICH) 7 November 1996 (1996-11-07) cited in the application page 42; claim 14; figure 6; example 11 --- -/--	1-3,5,6, 10-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2000	21/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Espen, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00870

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L,E	WO 00 55191 A (LOOSMORE SHEENA M ;CONNAUGHT LAB (CA); YANG YAN PING (CA); KLEIN M) 21 September 2000 (2000-09-21) L:priority SEQ ID NO 48,49	1,3,5,6, 10-24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
CA 00/00870

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9634960 A	07-11-1996	US 5808024 A	15-09-1998
		AU 722132 B	20-07-2000
		AU 5394196 A	21-11-1996
		CA 2219889 A	07-11-1996
		EP 0826052 A	04-03-1998
		JP 11502415 T	02-03-1999
WO 0055191 A	21-09-2000	NONE	